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Advancing the Applicability of Alternatives Assessment for Engineered Nanomaterials

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Society of Environmental Toxicology and Chemistry
7th SETAC World Congress
North America 37th Annual Meeting

Orlando, Florida | 6–10 November 2016

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abstract book

7th SETAC World Congress
SETAC North America 37th Annual Meeting

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This book comprises the abstracts of the presentations for the platform and poster sessions of the 7th Society of Environmental Toxicology and Chemistry (SETAC) World Congress/SETAC North America 37th Annual Meeting, conducted at the Rosen Shingle Creek in Orlando, Florida, 6–10 November 2016. The abstracts are reproduced as accepted by the Scientific Program Committee and appear in numerical order.

In each abstract, the presenting author's name is underlined. The author index cross-references the corresponding abstract numbers. Affiliation, session and keyword indices are also included.

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The Society of Environmental Toxicology and Chemistry (SETAC), with offices in North America and Europe, is a non-profit, professional society established to provide a forum for individuals and institutions engaged in the study, analysis and solution of environmental problems, the management and regulation of natural resources, environmental education, and research and development.

Specific goals of the society are:

- Promote research, education and training in the environmental sciences
- Promote the systematic application of all relevant scientific disciplines to the evaluation of chemical hazards
- Participate in the scientific interpretation of issues concerned with hazard assessment and risk analysis
- Support the development of ecologically acceptable practices and principles
- Provide a forum (meetings and publications) for communication among professionals in government, business, academia and other segments of society involved in the use, protection and management of our environment

These goals are pursued through the conduct of numerous activities, which include:

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- Publish scientific journals, a newsletter and special technical publications
- Provide funds for education and training through the SETAC Scholarship/Fellowship Program
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ENVIRONMENTAL QUALITY THROUGH SCIENCE®

Bioavailability of Organic Chemicals for Retrospective Risk Assessment: Measurement, Applications and Communication

1 Presentation of the newly standardized soil quality TRIAD approach, with special focus on the inclusion of bioavailability

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Recently, ISO standardized the performance of the TRIAD approach as a method to ensure the quality of the site-specific ecological risk assessment of soil contamination (originally, the TRIAD was developed for sediments). The norm ISO 19204 is based on experiences made in The Netherlands, Great Britain and Denmark as well as in other parts of the world (e.g. Brazil). The term TRIAD relates to three Lines of Evidence (LOE): chemistry, toxicology and ecology. Each of these LOEs is performed on three tiers (screening, refined screening, detailed assessment) with increasing complexity of the tests to be performed. In view of the nature of this International Standard the investigation procedure is described on a general level, but details of technical procedures for the actual assessment are given in related technical standards (e.g. ISO 15799, ISO 17616). In ecological risk assessment, the effects of soil contamination on the ecosystem are related to the intended land use and the requirements that this use sets for properly functioning soil. The ISO Standard describes the basic steps relating to a coherent tool for a site-specific risk assessment with opportunities to work out site-specific details. Inclusion of bioavailability improves the applicability of the TRIAD approach. In this contribution we will give a short overview on the soil quality TRIAD ISO standard, starting with simple approaches to link it with existing generic soil values (based on total concentrations) in the screening tier. Indicators for actual and potential bioavailability refine the tier. For organic chemicals passive sampling and Tenax/cyclodexrine are suitable tools comparable to 0,001 mol/l CaCl2 and 0,43 mol/l HNO3 for heavy metals. Because the two other LOE's (toxicology and ecology) are also directly related with bioavailability a more balanced TRIAD is obtained. Finally, on the detailed assessment level more complex methods might be used, such as chemical models, or the use of more site specific organisms. Advanced or complex methods and experimental designs are not excluded. Currently, practical experience with the soil quality TRIAD approach is still limited, but in the light of a growing tendency to skip the use of total concentrations in soil ecological risk assessment (e.g. in the context of prospective risk assessment) it is expected that this situation will change in the foreseeable future. However, this expectation depends highly on further legal developments.

2 Use of Equilibrium Passive Sampling Devices for Measuring Bioavailable PAH and Related Heterocyclic Compounds

K. O'Neal, CH2M; X.R. Xia, North Carolina State Univ / CCTRP/CVM; X. Kong, P. Lazaro, D. Shea, North Carolina State Univ

Passive sampling devices (PSDs) that are allowed to reach equilibrium with water or water-particle slurries have shown promise in estimating the readily desorbable and potentially bioavailable fraction of polycyclic aromatic hydrocarbons (PAHs). To make further progress toward this goal, we measured the equilibrium polymer-water partition coefficients for 85 PAHs and related heterocyclic compounds and for 27 oxygenated- and nitro-PAHs (O/N-PAH) using polyoxymethylene (POM) as a non-depleting sorbent phase. Our measured partition coefficients (KPOM) are in good agreement with previously published values for the USEPA "PAH-34" list and our data set extends the values to a much larger and more useful group of PAH and O/N-PAH degradation products. We used both a static-renewal water-only exposure and a sediment-water slurry exposure to measure KPOM and found good agreement between the two methods. Our measured log KPOM values were linearly correlated to log KOW ($r2 = 0.96$ for static-renewal experiment and $r2 = 0.93$ for sediment-water slurry). We then measured dissolved PAH in a sediment-water slurry, using PAH-contaminated sediment from the field, and compared the POM method to solid-phase microextraction (SPME) for measuring freely dissolved PAH. The POM provided data for 72 PAH and O/N-PAH compounds compared to only 3 PAH for the SPME owing to the much lower detection limits of the POM method. Furthermore, to provide toxicological relevance to our chemical measurements, we conducted experiments with oil and creosote contaminated sediment pre- and post-remediation and found, with some exceptions, that the PAH concentrations in sediment-water slurry measured using the POM was well correlated with accumulation in freshwater mussels, providing strong evidence that the POM method measures only the bioavailable fraction of PAH in the sediment.

3 Using Passive Sampling to Measure Site Specific Partitioning and Refine Sediment Risk Assessment

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PAHs and PCBs are listed as the chemicals of potential ecological concerns for South Wilmington Wetlands. The objectives of this study were to evaluate the site specific porewater concentration and sediment partitioning constants for PAHs and PCBs using polyethylene passive samplers, and to assess the site-specific bio-concentration factor (BCF) through PCB bioaccumulation in freshwater invertebrates. Results were used to refine the sediment preliminary remediation goals (PRG) based on two ecological receptors: toxicity to benthic invertebrates and bioaccumulation in the Spotted Sandpiper. Finally, a steady state cap design model was used to examine the performance of a soil cap, as a potential remediation approach. For PAHs, the site specific sediment partitioning constants (Koc) were 1-2 orders of magnitude higher than the generic values used in preliminary assessments, which resulted in higher revised PRGs than initial ones based on the protection of benthic invertebrates. The estimated PRG based on benthic exposure is more restrictive compared to exposure to the Spotted Sandpiper as PAHs do not bio-magnify and primarily pose problems for benthic invertebrates. For PCBs, nearly 1 order of magnitude higher site-specific Koc was observed than generic values. Bioaccumulation of PCBs in worms followed the typical behavior and was consistent with generic correlations for organism uptake, which could be largely explained based on PCB sediment porewater concentration. For PCBs, exposure to the Spotted Sandpiper resulted in a more restrictive PRG compared to benthos based PRGs. Overall, site-specific assessment of PAH and PCB partitioning in sediments was able to refine risk assessment for South Wilmington Wetlands and develop more realistic PRGs. Conventional estimation of porewater concentration using generic organic carbon partition constants was not predictive of bioaccumulation in worms. PRG derived from generic values could be orders of magnitude different from those based on site specific measurement. Also, the PRG for the same contaminant could be orders of magnitude different based on different receptors. Thus, selection of appropriate ecological receptor is crucial in risk assessment. Finally, the cap modeling results indicated that application of a soil cap with 5% organic carbon was able to achieve the revised remediation goals, by reducing (over 90%) of the sediment toxicity, and exposure of benthic and avian species to PAHs, PCBs and pesticides.

4 A probabilistic framework for evaluating the likelihood of toxicity in sediments based on site-specific partitioning and passive sampling methods

A.A. Brennan, Univ of Minnesota Duluth; N.W. Johnson, Univ of Minnesota Duluth / Civil Engineering

A probabilistic decision framework was developed to demonstrate how SPME-derived C_{free} measurements for PAHs can be used to evaluate the accuracy of risk management decisions in moderately contaminated sites where the toxicity of sediments is uncertain. The framework builds upon tiered assessment approaches by incorporating a measure of the likelihood that concentrations found at moderately contaminated sites are protective of benthic organisms. Results of a probabilistic analysis illuminate large variability in the effective partitioning values for total organic carbon and black carbon, and demonstrate that any single, deterministic partitioning prediction implicitly provides protection to some likelihood. Until standard passive sampling methods are widely accepted and implemented or partitioning behavior of PAHs is understood more fully and can accurately predict porewater concentrations for all sediments, a statistical framework for using site-specific SPME-derived K_{OC} partitioning distributions can provide a cost-effective, nuanced option for predicting risk in a range of sediment types. The proposed decision framework provides flexible statistical guidance for moving through tiered assessment approaches that incorporate 1) screening level targets of predicted interstitial water concentrations (C_{free}) from bulk sediment concentrations (C_{total}) and site-specific SPME-derived K_{OC} partitioning distributions 2) SPME-derived C_{free} estimates of bioaccumulation and toxicity and 3) actual biological (toxicity or bioaccumulation) testing. The decision framework was developed and applied in the context of data collected from a pilot project utilizing navigational dredged materials for creation of shallow water habitat in one of the Great Lakes Areas of Concern: the St. Louis River Estuary in Duluth, MN, USA. Results suggest that areas of moderate contamination stand to gain the most from direct measures of C_{free} during initial stages of assessment.

5 Development of Invertebrate Bioaccumulation Factors for Polychlorinated Biphenyls

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Predictive ecological risk assessments rely on numerous assumptions regarding the uptake of chemicals by receptors. Bioaccumulation of chemicals from soil to biotic tissue is a primary uncertainty when predicting exposure to receptors in the food web. In the absence of site-specific tissue data, an increased understanding of chemical bioaccumulation at lower trophic levels can greatly increase the predictive ability of ecological risk assessments to allow for more refined evaluation of potential ecological risk. When evaluating potential ecological risk to PCBs in soil, it is often necessary to derive estimates of PCB concentrations in invertebrates, such as earthworms. At the base of the trophic pyramid, estimates of PCB concentration in earthworms and other invertebrates affect predicted exposure and potential risk associated with PCBs at all trophic levels. Commonly used literature-based BAFs have been developed from PCB uptake data derived from limited laboratory and field datasets. However, these BAFs may not account for site-specific factors that can significantly alter uptake of PCBs such as congener composition, weathering, and site-specific abiotic or biotic factors. To evaluate the potential effect of these considerations on uptake estimates, we reviewed the literature on PCB bioaccumulation by invertebrates and assembled a database of PCB bioaccumulation data that included information regarding specific congeners or Aroclors, as well as weathering, biotic, and abiotic parameters. We used available data from sites with similar PCB structure and site conditions to model soil-invertebrate bioaccumulation. As it is not always feasible to obtain site-specific uptake data and develop site-specific soil to earthworm BAFs, the use of BAFs modeled for specific congeners and site conditions can reduce uncertainty and increase the predictive ability of ecological risk assessments.

6 Assessing Bioavailability of High Molecular Weight Hydrocarbons in Weathered Soils from Intra-aggregate Pore Size Distributions

S. Ghoshal, A. Akbari, McGill Univ / Civil Engineering

The assessment of bioavailability of hydrophobic organic contaminants (HOCs) to microorganisms is of great interest in a priori assessments of bioremediation rates and extents. Several different paradigms have been used to assess the microbial availability of HOCs and to estimate remediation endpoints. For example, the rate and extent of physicochemical mass transfer of HOCs from contaminated soils to the aqueous phase have been commonly used to assess bioavailability, because it is generally understood that only aqueous phase contaminants are bioavailable to microorganisms. However, poorly soluble high molecular weight organic compounds, such as those present in certain fractions of petroleum liquids, are often degraded only by direct contact of bacteria with the oil-water interface or by micellar solubilization and/or emulsification by biosurfactants excreted by bacteria near oil-water interfaces. In weathered petroleum-contaminated soils, a significant fraction of poorly soluble hydrocarbons reside in sub-micron sized pores of soil aggregates. Given that the bacteria are typically larger than these pores, direct contact with those hydrocarbon fractions are not expected, and hydrocarbons present in larger pores are not considered as bioavailable. We have developed a X-ray microcomputed tomography technique, supplemented by gas adsorption and mercury porosimetry measurements, that can be used to characterize a broad range pore size distributions in soil aggregates to enable estimation of bioaccessible pore volumes. Our recent investigations however suggest that an approximate size of bacteria may results in incorrect estimation of microbial bioavailability. Our detailed experimental studies demonstrate that certain growth conditions and bacterial properties cause aggregation of bacteria, which results in bacterial entry only into pores 5 microns and larger; while in situations where there is active bacterial growth near pore mouths, bacteria in early growth stages can enter pores as small as 0.4 microns. These findings provide a basis for estimating bioaccessible pore volumes which can then be used towards rational assessments of bioremediation endpoints in porous soil aggregates.

7 Are depletive and passive samplers interchangeable exposure metrics to estimate interstitial water concentration?

S. Nutile, Southern Illinois Univ Carbondale / Zoology; A. Harwood, Alma College / Environmental StudiesBiology; F.L. Sinche, K. Huff Hartz, Southern Illinois Univ Carbondale / Center for Fisheries Aquaculture and Aquatic Sciences; P.F. Landrum, Southern Illinois Univ; M.J. Lydy, Southern Illinois Univ-Carbondale / Center for Fisheries Aquaculture and Aquatic Sciences

It is well recognized that defining bioavailability is essential for accurately estimating exposure in risk assessments. Bioavailability- and bioaccessibility-based metrics, such as passive samplers and Tenax extractions, respectively, provide accurate exposure estimates of contaminants in sediments by representing the chemical fraction that is, or will, become available for biological uptake. Use of passive samplers under equilibrium conditions is time consuming, but use under non-equilibrium conditions requires modeling and extrapolation. Additionally, defining the bioaccessible fraction through estimates of the rapidly desorbing fraction (F_{rap}) with sequential Tenax extractions can take days to weeks. Single-point Tenax extractable concentrations, however, represent F_{rap} and provide accurate estimates of bioaccessibility. As the link between Tenax extractable concentrations and interstitial water concentration at equilibrium (C_{IW}) is not well defined, few risk assessments utilize this method. This study examined the relationship between bioavailability- and bioaccessibility-based techniques by comparing F_{rap}, SPME fiber concentrations, and 24 h single-point Tenax extractions by using estimates of C_{IW} as a comparative media. Comparisons of SPME fibers at equilibrium to F_{rap} estimated from full desorption curves by calculating C_{IW} yielded a strong linear regression, suggesting both bioavailability and bioaccessibility are driven by F_{rap}. Since single-point Tenax extractions are more convenient than quantifying F_{rap} using full desorption curves or using SPME fibers at equilibrium, comparisons of C_{IW} calculated using single-point Tenax extractions and SPME fibers were made to understand if estimates of F_{rap} provided by single-point extractions were comparable to full desorption curves and passive samplers. A significant linear regression was found, demonstrating that 24 h single-point Tenax extractions provide operationally defined estimates of C_{IW} at equilibrium similar to that of passive samplers. The slope of this regression line, however, is dependent on the length of the Tenax extraction, as 24 h extractions represent desorption from F_{rap} as well as the slow and very slowly desorbing fractions. Therefore, both bioavailability- and bioaccessibility-based exposure metric estimates are controlled by F_{rap}, provide similar estimates of C_{IW} at equilibrium, and as such, should be considered complementary tools for use in risk assessments.

8 Single-point Tenax extractions: An effective but underutilized tool in sediment risk assessments

A. Harwood, Alma College / Environmental StudiesBiology; S. Nutile, Southern Illinois Univ Carbondale / Zoology; F.L. Sinche, K. Huff Hartz, Southern Illinois Univ Carbondale / Center for Fisheries Aquaculture and Aquatic Sciences; P.F. Landrum, Southern Illinois Univ; M.J. Lydy, Southern Illinois Univ-Carbondale / Center for Fisheries Aquaculture and Aquatic Sciences

Numerous studies have utilized the single-point Tenax extractable concentration to evaluate bioaccumulation potential of hydrophobic organics. Ultimately, the Bioaccumulation Tenax Model, which relates the 24 h Tenax extractable concentration to lipid normalized tissue concentrations, was developed. This model now contains over 750 data points, representing several compound classes and species. Despite the apparent robustness of this model, Tenax is still not a widely recognized method for sediment assessments. One reason for this is the limited information on the consistency of this method across sediments, experimental methodologies, and compound classes. The objective of the current study was to evaluate which variables can influence the consistency and applicability of Tenax extractable concentrations to evaluate bioaccumulation. We determined that in most cases the Tenax extractable concentrations were consistent regardless of Tenax to organic carbon ratios or Tenax mass. The relationship between the 24 h Tenax extractable concentration and the rapidly desorbing fraction also was unaffected by Tenax to organic carbon ratios, hydrophobicity, organic carbon content of sediments, and only slightly impacted by aging time. However, while Tenax extractable concentrations can be related to mortality, caution is advised when using Tenax extractable concentrations to estimate bioaccumulation when sediments are toxic; therefore, selection of appropriate endpoints is essential to optimally utilizing this technique. This further demonstrates that 24 h single-point Tenax extractions are a robust method for evaluating bioaccumulation potential in sediments and are a powerful underutilized tool for risk assessments of hydrophobic organics.

Uncharted Waters: Field Ecotoxicology in Remote Locations on Limited Resources

9 An Ecotoxicological Voyage into Central Asia

D.D. Snow, Univ of Nebraska / School of Natural Resources; A.S. Kolok, Univ of Nebraska – Omaha / Nebraska Watershed Network; S. Bartelt-Hunt, Univ of Nebraska-Lincoln / Civil Engineering; B. Uralbekov, al-Farabi Kazakh National Univ; N. Mamilov, al-Farabi Kazakh National Univ / Biology and Biotechnology; M.K. Sellin Jeffries, Texas Christian Univ / Dept of Biology

When conducted in developing countries, ecotoxicological research may be particularly challenging. Here, we present lessons learned from a recent reconnaissance mission to Kazakhstan to highlight such challenges and offer potential solutions. In our experience, the researcher is likely to confront an environment in which there is little technical or logistic support for field work. Furthermore, extensive and sophisticated sample collection, preparation and processing that field scientists typically enjoy in developed countries is often not possible. Even the taken for granted background information, such as maps and traveling details, may either be unavailable or available only in a language and format that dramatically limits utility. Due to these limitations, it is important to plan ahead early, and to incorporate the expertise of locals into the travel plans and coordination. If the travelers include those who have not experienced the culture, landscapes and uncertainty of an expedition to a foreign country, then it is also critical that group members be continually involved in the planning and inevitable change of plans. With respect to data collection, it may be important to collect data that encompasses trace level organic and inorganic contaminants, as well as biological tissues and field measurements, particularly if the expected contaminants are uncertain. In spite of the risks, the rewards of a successful field expedition to a foreign country are considerable and the lessons learned will ultimately pave the way to better understanding of how to best manage aquatic ecosystems both for a growing global community and shrinking planet.

10 Documenting water quality in the Syr Darya River Basin – the utility of passive sampling

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The Syr Darya River originates in the Tian Shan Mountains in Kyrgyzstan and eastern Uzbekistan and flows through southern Kazakhstsan to what remains of the Aral Sea. The river basin in Kazakhstan and Uzbekistan is an area of intensive agricultural production, and annual use of pesticides and insecticides in the lower region has been estimated at 34,000 to 52,000 metric tons. In June and October 2015, we conducted a sampling campaign along the Syr Darya to evaluate the occurrence of pesticides, insecticides, metals and radionuclides to better eludicidate the water quality impacts from intensive agricultural practices and mining activity in the region. A variety of metals were detected including vanadium, uranium, iron, copper and zinc, generally showing increasing concentrations with distance downstream. Analysis of water and sediment samples demonstrated the occurrence of lindane, trifluralin and residues of legacy pesticides including 4,4-DDE. Passive organic chemical integrative samplers (POCIS) were deplyed in the Syr Darya. A variety of compounds were detected in both grab samples and POCIS including neoicotinoid and organophosphorus insecticides, as well as triazine and chloroacetanilide herbicides. Though a POCIS was lost at one location, a larger number of compounds were detected in the POCIS extracts when compared to the grab samples, indicating the potential utility of passive sampling in remote locations or when contaminant concentrations are not well understood.

11 An ecotoxicological reconnaissance in Central Asia: Assessment of biomarker responses in wild-caught roach (Rutilus rutilus)

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With increasing global demand for agricultural products, countries of the developing world, such as Kazakhstan, have been pressured to implement strategies for intensive agricultural production. Some strategies have led to a variety of environmental disasters, one of the worst being the recent dewatering of the vast Aral Sea. Water diversion from the Syr and Amu Darya rivers since the 1950s to supplement agricultural production in the arid region has not only caused the subsequent dewatering of the Aral Sea, but has led to the wholesale collapse of the Sea's historically important fishery. Recent investigations have revealed the residues of both modern and legacy pesticides (e.g. 4,4-DDE, a metabolite of the pesticide DDT), heavy metals (e.g. vanadium), and radionuclides in the Syr Darya watershed, potentially arising from agricultural runoff, mining and changing land uses. The goal of this reconnaissance project was to assess the biological implications of contaminants in the Syr Darya watershed. This was accomplished by taking physical measurements (i.e. mass, length, condition factor, GSI, and HSI) and tissue (i.e. liver, brain, gonad, gill) samples from roach fish (Rutilus rutilus) at three sites along the Syr Darya. Tissue samples were analyzed for genes considered to be biomarkers of chemical exposure. Quantitative PCR analysis of these tissues revealed the altered expression of genes perceived as indicators of a generalized stress response (e.g. heat shock protein 70, superoxide

dismutase), and xenobiotic metabolism (e.g. cytochrome p450 enzyme 1A). The implications of these findings will be discussed in the context of land use and chemical residues. In keeping with the session's theme, we will also discuss some of the difficulties and resolutions associated with collecting biological samples in remote regions of Kazakhstan.

12 Development of a native pencil catfish (Trichomycterus areolatus) as a freshwater environmental sentinel of pollution in Chile

J.M. Ali, Univ of Nebraska Medical Center / Environmental, Agricultural and Occupational Health; A.S. Kolok, Univ of Nebraska – Omaha / Nebraska Watershed Network

Much of the country of Chile is a desert environment with unique and challenging problems relative to the discipline of ecotoxicology. Here, the rivers run a relatively short distance from their origins in the Andean Mountains to the Pacific Ocean and are separated by miles of desert. Despite their remote locations in the Andes many of these river systems are subject to intensive utilization by mining, industry and agriculture that contribute to their degradation. We have identified a native environmental sentinel for investigating pollutants in the scarce freshwater ecosystems of Chile. Trichomycterus areolatus, is a native pencil catfish found in streams throughout Central Chile and is an ideal candidate for a sentinel organism due to its relative abundance and intimate interaction with both the sediment-water interface. We sequenced the adult transcriptome of T. areolatus using tissue samples collected from the Choapa River in Coquimbo (Region IV), Chile. Based on this data, we identified differences in hepatic gene expression downstream of pollution sources along the Choapa River in January 2015. Among other site specific changes in hepatic gene expression, we found defeminization of female specimens collected downstream of areas with intense agricultural activity. Additionally, male specimens collected near the city of Salamanca showed an upregulation in hepatic expression of CYP1A. However, challenges remain with implementing this species as an environmental sentinel including a lack of knowledge about its reproductive strategy, typical feeding behaviors and life history.

13 Vitellogenin detection and quantification in blood plasma and surface mucus of a range of Australian freshwater and marine fishes

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Research on the impacts of endocrine disrupting chemicals (EDCs) to Australian fishes is restricted to just a few studies due largely to a lack of tools, resources and limited local expertise. Australia, like many other locations that have limited ecotoxicology resources, also exhibit harsh environmental conditions (ie. long periods of drought) and have unique flora and fauna. For these reasons, research to establish the sensitivities of local, native species is necessary to determine realistic estimates of risks from EDCs in these environments. However, there are currently no specific tests for Australian species, which, combined with an underlying lack of fundamental knowledge of the reproductive physiology in most native fishes, creates challenges when trying to measure endocrine disruption in environmental monitoring and laboratory-exposure studies. Measurement of the yolk-protein precursor, vitellogenin (VTG) is a widely used and well established biomarker for assessment of exposure to estrogenic endocrine disrupting chemicals. Globally, the induction of VTG in juvenile and male fish is used in standardised testing guidelines (eg. OECD, USEPA) to infer exposure to sewage-derived estrogens and other xenoestrogens (eg. Bisphenol A, nonylphenol), in well described, model species such as zebrafish, fathead minnows, carp, roach and rainbow trout. The measurement of VTG is predominantly carried out by enzyme-linked immunosorbent assay (ELISA), using antibodies that have been developed for specific test species, however, the production of VTG antibodies is costly, time consuming and has animal ethics considerations and is therefore not a viable option for development in multiple Australian fish species. Here we report the use of two commercially available ELISA kits for quantitative measurement of VTG in blood plasma and surface mucus of several fish species sampled from rivers and estuaries around Melbourne, Australia. Successful testing has been done on native species from several fish families, including Sparidae, Tetraodontidae, Percichthyidae, Gobidae and Eleotridae, as well as introduced species from Cyprinidae and Percidae. This study demonstrates the utility of applying existing tools to non-standard test species in order to progress research into endocrine disruption impacts in Australia, despite the lack of local tools and resources.

14 Grocery Store Ecotoxicology: Mercury in food fish from two major river systems in Colombia

A.S. Kolok, Univ of Nebraska – Omaha / Nebraska Watershed Network

It is not uncommon for modern mining to occur in remote regions of the world. One such region is the Nechi River near El Bagre in northeastern Colombia. The Nechi River watershed is known for its gold deposits and both artisanal and industrial mining operations extract the resource from the river sediments. A considerable amount of elemental mercury is used in the extraction process, and there is concern that mercury contamination can occur both occupationally and environmentally. Relative to environmental contamination, it is possible that large predatory fish, such as whiskered catfish, can biomagnify mercury in their muscle making the meat toxic if consumed. Efforts to sample large fish from the local rivers are thwarted by the lack of reliable transportation locally and by the limitations in the type of equipment that can reliably be transported to such remote regions. Under these conditions, a successful strategy that can be employed is to sample fish collected for human consumption, either by the local fishers as they bring their catch to market or directly from the market place itself. Using this strategy, we attempted to determine whether fish from the Nechi River were more contaminated with mercury than the nearby La Miel River, a tributary of the Magdalena River where there are no pronounced gold deposits. Six different fish species were collected from each river system and in general the fish from the Nechi had higher levels of mercury in their tissues than did those from La Miel. More importantly, the levels of mercury found in the Nechi were highest in muscle tissue from two different species of Pimelodid, or whiskered, catfish. It seems likely that species from the Pimelodid family can be used as environmental sentinel organisms relative to mercury contamination. Furthermore, it seems plausible that fish collected at local fish markets can be very useful to evaluate mercury contamination on a watershed scale.

15 Challenges in assessing chemical contamination of water in resource poor countries: Lessons learned during field sampling in Haiti

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The large majority of outreach efforts in resource poor countries are focused on infectious diseases that plague these areas. But unregulated chemical use and discharge, and inadequate treatment infrastructure may also lead to chemical contamination risks to aquatic organisms as well as animals and humans that use contaminated water sources for drinking, cooking, and cleaning. Additionally, many chemical contaminants are known to exacerbate infectious disease, which may contribute to the epidemic disease outbreaks around the world. Yet little work has been done to characterize the presence and potential environmental health risks of aquatic chemical contaminants in resource poor countries. Haiti is located on the island of Hispaniola and is recognized as the poorest country in the western hemisphere. It has been plagued with widespread environmental health issues resulting from poor infrastructure, little regulation, lack of education, and a receding economy. In Haiti, little attention has been paid to chemical contaminants in aquatic systems, therefore, our team set out in the spring of 2015 and 2016 to conduct small scale surveys of contaminants in surface and subsurface waters. Two techniques were implemented to assess the presence of organic

contaminants: high throughput hormone receptor bioanalytical assays for screening of endocrine activity and non-target mass spectrometry for identification of chemicals present in these waters. Initial results from these assays indicate the presence of low levels of numerous chemical contaminants including estrogenic chemicals, pesticides, pharmaceuticals, and plasticizers. Results from these studies will be used to prioritize future sampling and be matched with future exposure assessments to inform risk analysis for Haitian populations. Many challenges were encountered during these studies including import/export, logistical, and infrastructure issues that will be addressed during this presentation. Lessons learned from our work in Haiti are useful as toxicological assessments in underdeveloped settings gain traction in support of improving ecosystem and human health.

16 Ecotoxicological Research in Remote and International Settings: Challenges and Solutions

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Ecotoxicological research in remote and/or international settings is fraught with challenges and rewards. However, research efforts in these locales is critical to establish the effects of contaminants on both human and environmental health. We will discuss lessons learned from three investigator perspectives including both obstacles and solutions while working in remote areas around the world. Establishing collaborations across distance and culture involves language, technical, legal, and cultural challenges. Working with local collaborators with culturally relevant experience is critical. Second, training in cross-cultural sensitivity relevant to the research site, as well as basic language skills, build foundations with local research partners and the community. Gaining the confidence of the support people on site is fundamental to navigating the regulatory rules involving transportation, permitting, and sample collection. Planning needs to include concrete emergency preparation for potentially life threatening situations such as infectious diseases, political instability, catastrophic events, and dangerous animals. Building a knowledge-base and support system requires site visitation, collaborator and support staff meetings, and evaluation of field conditions. Site visitation also enables the collection of preliminary data and the identification of suitable model species necessary for successful grant applications. Creative funding solutions often require combinations of support from diverse granting resources to promote project sustainability; grant resource examples include non-governmental organizations, international aid agencies, local and federal grant programs, and the home institution. A challenge for the research community is educating funding agencies regarding the hurdles and need for ecotoxicological research in remote locations and developing countries. Collectively these approaches will facilitate research and therefore broaden our understanding of the impact of pollution on a global scale.

Exposure, Effects and Fate of New Organic Contaminants in Aquatic Ecosystems

17 Advances in the fugacity modeling of emerging substances in aquatic ecosystems

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Fugacity modeling was proposed by Don Mackay as a comprehensive framework to model the fate of substances in the environment. In fugacity modeling virtually all important processes can be included: (1) partitioning, (2) bioaccumulation, (3) degradation and synthesis reactions, (3) advection and diffusion, (6) loadings to the environment, and many other processes. Since its inception, the application of fugacity modeling has evolved from simpler to more complex systems. Early fugacity models described processes on a continental scale. Later models moved to smaller units such as lakes, drainage basins and watersheds. Other fugacity models have been developed to characterize processes such as wastewater treatment and the land application of biosolids. In recent years we have applied fugacity based models to the prediction of the ecological risks due to the disposal of consumer products and substances of emerging concern. Substances such as triclosan and bisphenol A have been recently evaluated to characterize risks to aquatic systems. Earlier models utilized WWTP models, watershed scale fate and transport models to predict the steady state concentrations in abiotic media, and food web based bioaccumulation models to predict dietary and tissue concentration. In an effort to more accurately capture geographic variability we have moved to smaller model units, and more recent models have been reparametrized to link the units together using a flow network. The flow network links upstream model units to downstream model units and more faithfully simulates the movement of substances through a drainage basin. We have also developed additional model algorithms that not only incorporate the degradation of substances but also include the fate of these degradation products in the simulations. The primary challenges to effective fugacity modeling are (1) the accurate estimation of chemical characteristics such as K_{oc} and media specific degradation rates, (2) pathway specific loadings of point and diffuse sources to abiotic media, and (3) the characterization of the model units including the volume of surface water, surface soil, and sediment, surface water flow and flow pathways, and the boundaries of each model unit. The use of these models provides a means by which the risks associated with substance can be assessed in the absence of comprehensive monitoring data.

18 Temporal trends of PBDEs and emerging flame retardants in endangered beluga whales from the St. Lawrence Estuary (Canada)

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Located downstream of several large cities, the St. Lawrence Estuary (Eastern Canada) has over the years become a highly contaminated area by halogenated flame retardants (HFRs). Each summer, many whale species migrate into the St. Lawrence Estuary for feeding. Due to their high trophic level, certain toothed and baleen whales from this area are highly exposed to HFRs including polybrominated diphenyl ethers (PBDEs) and several other HFRs. A study reported a significant increase in blubber PBDE concentrations in stranded beluga whales (Delphinapterus leucas) from the St. Lawrence Estuary between 1987 and 1997, followed by a plateau up until 2007. Although St. Lawrence belugas have been protected from hunting since 1979, this population has not recovered and was reclassified as endangered in 2014. This might be partly explained by high exposure to HFRs and other contaminants. We investigated the variations of 35 PBDE congeners and 12 emerging HFRs (e.g., Hexabromobenzene (HBB) and dechlorane-related compounds) in the blubber of 50 stranded male belugas found along the Estuary and Gulf of St. Lawrence between 1997 and 2013. We also examined the occurrence of these HFRs in 11 stranded male and female minke whales (Balænoptera acutorostrata) from the St. Lawrence as well as 6 male belugas from Nunavik (Canadian Arctic) collected as part of the traditional hunt. PBDEs were the most abundant HFRs in all three whale populations and 6 emerging HFRs were quantified in the majority of samples. ΣPBDE concentrations in blubber of St. Lawrence belugas were 3- and 5-fold greater than minke whales and Arctic belugas, respectively. Overall, concentrations of emerging HFRs in belugas from the St. Lawrence were notably greater compared to the two other populations, with the exception of Dechlorane plus (DP) and Dec-604 Component B that were greater in Arctic belugas. No significant trend of PBDEs was found in St. Lawrence belugas during this 17-year period. In contrast, concentrations of HBB and Chlordene plus decreased slightly from 1997 to 2013, while DP increased up until 2000 and decreased slightly thereafter. Age and diet (i.e., stable carbon and nitrogen isotope signatures) of whales did not influence HFR concentrations. Despite several years of PBDE regulations, blubber levels of PBDEs in St. Lawrence belugas have remained unchanged. The occurrence of alternative HFRs in these cetaceans warrants continuous monitoring of these substances.

19 Mass Balance Study of Lake Michigan for Organic Pollutants in 2010-2015

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This study revisits and updates the Lake Michigan Mass Balance Study that was conducted by federal, state, university, and private-sector researchers between 1994 and 1996. The current work combines recent data from Lake Michigan tributary water, air, open lake water, and sediment to calculate an updated mass balance for polychlorinated biphenyls (PCBs), brominated flame retardants (BFRs), and organophosphate esters (OPEs). The fluxes or loads of tributary, open lake water, air, and sediment sources were calculated and compared to produce an approximate present-day mass balance. Water samples, including both the dissolved and particle phases, were collected every three weeks in 2015 from the Grand, Kalamazoo, St. Joseph, and Lower Fox Rivers and from the Indiana Harbor and Ship Canal. Air samples, including both particle and vapor phases, were obtained from Chicago and Sleeping Bear Dunes every 12 days as part of the United States' Integrated Atmospheric Deposition Network (IADN). Open lake water samples were previously analyzed in the Hites laboratory in conjunction with Environment Canada. Sediment data were obtained from the Great Lakes Sediment Surveillance Program. Tributary sample concentration ranges in 2015 for PCBs, BFRs, and OPEs were 0.5-50 ng/L, 0.2-3.7 ng/L, and 0.4-150 ng/L, respectively. Significant differences in mean concentrations were found among the five rivers; the Indiana Harbor and Ship Canal had the highest mean concentrations for the three groups of compounds. Preliminary results suggest that the Chicago remains the main source of atmospheric deposition to Lake Michigan for many of these compounds. The concentrations of PCBs and some BFRs measured in our study were less than that measured/computed by previous studies, possibly indicating a downward trend.

20 Linear and cyclic volatile methylsiloxanes in blood of freshwater and marine piscivorous wildlife from Canada

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Cyclic and linear volatile methylsiloxanes (VMS) substances are widely used as ingredients for cosmetic and personal care products including anti-perspirant, baby products, shampoo, nail polishes, etc. Blood of piscivorous wildlife (snapping turtles, double-crested cormorants, and grey seals), was collected in Canadian freshwater and marine ecosystems. A modified quick, easy, cheap, effective, rugged, and safe (QuEChERS) method was developed to measure VMS in blood, using large-volume injection with gas chromatography-mass spectrometry (LVI-GC-MS). Cyclic VMS hexmethylcyclotresiloxane (D3), octamethylcyclotetrasiloxane (D4), decamethylcyclopentasiloxane (D5), and dodecamethylcyclohexasiloxane (D6) were present in blood of all three species. Linear VMS were only found in seal blood in one contaminated site. For most animals, D5 was the dominant compound, with mean concentrations ranging between 0.143 and 7.39 ng/g ww. Further, D5 showed the largest spatial variation for each species, as all showed elevated D5 concentrations in contaminated sites compared to the reference sites. Our data indicated that local urban sources of VMS contribute significantly to the observed D5 concentrations in free ranging wildlife. Given that the blood:air partition coefficient of VMS is low but the lipid:water partition coefficient is very high, diet is likely the main source of VMS to piscivorous wildlife. The presence of cyclic VMS in the blood of three species from different taxonomic groups in marine vs freshwater ecosystems demonstrates that these chemicals are ubiquitous in aquatic biological systems.

21 Scenario analysis of the persistence of linear and cyclic volatile methylsiloxanes in aquatic environments using multimedia models

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Volatile methylsiloxanes (VMS) are a group of organosilicon chemicals that consist of SiO-chains in cyclic or linear ordinance and have methyl groups attached to the silicon atoms. Because of their widespread use in personal care products, cyclic and linear volatile methylsiloxanes (cVMS and lVMS) have been found at considerable levels in air, surface waters, sediments and aquatic organisms. In surface waters and sediments, the residence times of VMS are primarily controlled by their sorption to organic carbon, characterized by their organic carbon/water partition ratio (K_{OC}). Kozerski et al.[1] reported a log K_{OC} value of 5.17 for decamethylcyclopentasiloxane (D_5) and in a previous study[2] we measured log K_{OC} of 6.12. To explore the implications of such differences in partitioning behavior, we modeled the fate of cVMS and lVMS in generic scenarios using the equilibrium criterion model (EQC) and in a regional scale scenario in Adventfjorden, Svalbard, Norway. Using the K_{OC} measurements of Kozerski et al.[1] in the EQC model the overall residence time for D_5 was 203 d, while using the K_{OC} measurements of Panagopoulos et al.[2] the overall residence time was more than 3 times longer at 676 d. Similarly, in the regional scenario when using a log K_{OC} of 5.17 the overall residence time for D_5 was 225 d, while using a log K_{OC} of 6.12 the overall residence time was 534 d. The one log unit higher K_{OC} substantially increased the residence times of D_5 such that their residence times in sediment exceeded the persistence (P) criterion for sediment set by REACH and the 100-day criterion for overall persistence suggested by Webster et al.[3] The modeling results underline the large influence of K_{OC} when estimating the persistence of VMS in aquatic environments.

(1) Kozerski et al. Environ. Toxicol. Chem., 2014, 33, 1937-1945. (2) Panagopoulos et al. Environ. Sci. Technol., 2015, 49, 12161–12168 (3) Webster et al. Environ. Toxicol. Chem., 1998, 17, 2148-2158

22 Polyhalogenated carbazoles in sediments of the Great Lakes: natural or anthropogenic?

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Aromatic organohalogens may originate from both natural and anthropogenic sources, and can be either detrimental or beneficial to the ecosystem and humans. To date, synthesized persistent and bioaccumulative organohalogens have received most attention in environmental monitoring. Evidence is mounting that known anthropogenic organohalogens compose only a small fraction of the total organohalogens found in the environment. In recent years, increasing detection of polyhalogenated carbazoles (PHCZs) has caused concern. PHCZs resemble polyhalogenated dibenzofurans in chemical structure with a planar molecular conformation that promotes toxicity. As such, PHCZs are termed nitrogenated dioxins and are considered as novel persistent organic pollutants. The sources of PHCZs found in the environment remain unknown and have been debated. The objective of this work was to gain insights into the origin of PHCZs found in natural waters. Ponar grab and core sediment samples were collected from the Great Lakes of North America, including Lakes Michigan, Superior, Huron, Erie and Ontario. The samples were analyzed and characterized for PHCZs using gas chromatography coupled with single- or triple-quadrupole mass spectrometry. The total accumulation of 26 PHCZs in the sediment is >3,000 tonnes, which exceeds those of known anthropogenic organic pollutant groups by orders of magnitude. Spatial and temporal patterns differ significantly among PHCZ congeners and among lakes. Based on their spatial and temporal trends as well as principle component analyses, both natural and anthropogenic sources are present. In particular, the majority of PHCZs found in the sediment of Lake Michigan is believed to have formed naturally. A number of "emerging" PHCZs deserve further monitoring as their deposition to the sediment has been increasing in recent decades. Lower substituted PHCZs may form from in situ dehalogenation of those with more halogens. Anthropogenic sources appear to exist, particularly for the emerging and some light molecular mass congeners.

23 A Chronological Record of Organophosphate Ester Deposition in Sediment

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Organophosphate esters (OPEs) are used as flame retardants and plasticizers in a variety of products. OPEs have been measured in surface sediments, however, their temporal trends , inferred from dated sediment cores, have not previously been determined. In this study, OPEs were determined in surface sediments from depositional zones of Lake Ontario and from Charleston Harbor area from South Carolina. Twelve of 22 OPEs were detected with >60% frequency in Charleston harbor sediments with tris (2-chloroisopropyl) phosphate (TCPP; 12 ng/g dry wt), tris(n-butyl) phosphate (TnBP; 13 ng/g dw) and ethylhexyl-diphenyl phosphate (EHDPP; 8.7 ng/g dw) predominating. TCPP and TnBP also represented a major proportion of total OPEs in surface sediments from a core collected from the urban influenced western basin of Lake Ontario but were less prominent in more remote central lake site. Analysis of dated cores from the two Lake Ontario sites showed exponential increases in depositional fluxes of 6 OPEs, including TCPP and TnBP, starting post-2000, possibly due to their growing use related to the phase out of polybrominated diphenyl ethers. Other OPEs had parabolic deposition profiles in the central basin suggesting maximum deposition in the 1990s. This is also the first chronological depositional analysis of OPEs in sediment cores in the Great Lakes and suggests emissions to aquatic environments are increasing.

24 Transcript, protein, and metabolite responses in rainbow trout (Oncorhynchus. mykiss) exposed to TBBPA-DBPE

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The Chemical Management Plan (CMP) has identified Tetrabromobisphenol A bis(2,3-dibromopropyl ether) (TBBPA-DBPE) as a replacement flame retardant with the potential to enter the Canadian aquatic environment. There is concern that TBBPA-DBPE might also pose a risk of disrupting the endocrine systems of exposed organisms. The present experiment was designed to assess the sub-lethal effect(s) of TBBPA-DBPE in juvenile rainbow trout in order to identify potential sub-lethal mechanistic pathways. The fish were exposed to waterborne TBBPA-DBPE in a flow-through design. TBBPA-DBPE was delivered to the tanks by peristaltic pumps to maintain consistent concentrations. Nominal exposure concentrations were: 0 µM ; 0.001-; 0.01-; 0.032-; and 0.1-µM. There were three separate exposures; one for 2 days, another for 10 days, and the last for 21 days. Eight fish were housed per tank, with three replicate tanks per exposure concentration for a grand total of 225 fish. We performed analysis of liver transcripts from fish exposed for 21 days, as well as shotgun analysis of plasma proteins and targeted analysis of plasma metabolites on fish from all exposures. We shall present a synthesis of the data from our multi-omics approach to gain insights onto the potential mode of action of TBBPA-DBPE in fish.

Environmental Chemistry

25 Two dimensional 1H and 13C-NMR characterization of atmospheric size segregated organic aerosols

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Atmospheric aerosol is a complex mixture of primary and secondary particles originating from anthropogenic and biogenic sources. Organic aerosols (OA) constitute from 50 up to 80% of atmospheric aerosol and play a very important role in the global climate acting as cloud condensation nuclei, atmosphere's oxidative burden and human health. Despite its significance, only 20% of OA is chemically characterized. In a recent review paper (Environmental Pollution, 2014,191, 232-249), we, for the first time, synthesized and critically evaluated the findings of NMR studies on OA, identified knowledge gaps and areas of research. These key issues included: optimization of sampling and analysis protocols, structural characterization of aqueous OA / humic-like substances and source apportionment. Proton and Carbon-NMR spectroscopy is an emerging method used to characterize environmental exposures to specific types of organic compounds. We applied 1D ^{1}H and 2D mononuclear ^{1}H-^{1}H, and heteronuclear ^{1}H-^{13}C NMR to determine the chemical composition of the water-soluble fraction of size segregated organic aerosol collected with a 5-stage (plus backup) cascade impactor at the following size intervals: < 0.49, 0.49-0.96, 0.96-1.5, 1.5-3.0, 3.0-7.2 and 7.2-30 µm. In this paper we discuss the functional group composition and source markers content of size-segregated aerosols, and the size distribution of biogenic and anthropogenic organic markers. Characteristic signals attributed to humic substances, carbohydrates, polyols, aromatic and aliphatic carboxylic acids were found with different contributions for each size fractions. Differences in the nature and contribution of sources, including soil, traffic exhausts and biomass burning, and in atmospheric processes during the same season were also revealed by functional group characterization.

26 Analysis of air monitoring data of cyclic volatile methylsiloxanes

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Due to their heavy use, cyclic volatile methylsiloxanes (cVMS) are under increased regulatory and scientific scrutiny. In last few years, the fate, transport and distribution of these compounds have been studied both by multimedia environmental fate modeling and also by the environmental monitoring. The objective of this project was to compare the air monitoring data of cVMS with their model predictions and to identify the critical data gaps in our understanding of the major environmental processes regulating the transport and distribution of cVMS in the global atmosphere. The published modeling assessments included both simple steady state simulations and time variable analysis at the global scales using both hypothetical and realistic release scenarios. The air environmental monitoring data are from Northern American and European including sites heavily impacted by human activities and the remote Arctic locations. The temporal and spatial patterns of cVMS concentrations in air were generally in a good agreement with the modeling predictions. However, the spatial gradient of air concentration of all three cVMS were much steeper than the spatial gradients predicted by the models. The biggest inconsistency with the model predictions was the spatial trends in the concentration ratios of various cVMS (e.g. D5/D6) measured at different locations. Further analysis suggested that the above discrepancy between the field data and model predictions could be attributed mainly to the possibility of the additional degradation mechanism in air that has not been recognized and incorporated in any of the current modeling assessments.

27 Use of Cholic Acid as a Surrogate Internal Standard for Steroid Hormone Quantitation in the Whale Blubber Matrix

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The International Whaling Commission Scientific Committee has stressed the critical need for studies on reproductive status of the western gray whale (Eschrichtius robustus) population, which it currently designates as critically endangered. Biopsy samples from western gray whales off Sakhalin Island have been collected in collaboration with Russian scientists since 2011. These samples provide precious material amenable to hormone, genetic or stable isotope analyses as previously reported (Gendron et al. 2015, Bickham et al. 2015, Bickham et al. 2013). Yet, with the current ELISA methodology to quantitate steroid hormones from the blubber matrix, limitations include challenges associated with the small amount of biopsy tissue available and cross-reactivity within the assay. Here, we report on a development of the quantitation of progesterone, testosterone, and cortisol via LC-MS/MS analysis in gray whale blubber. Samples from stranded eastern gray whales collected in 1997-2011 were homogenized, extracted for steroids, filtered, evaporated, and dissolved in 50 µL of a 60:40 solution of LC-MS grade water:acetonitrile with 0.1% formic acid. Samples were spiked with a surrogate internal standard of 5 nM cholic acid for the purposes of quantitation, diluted 1:10, and 1 ul was injected onto a C18 reversed-phase column. A 300 nL/min flow rate was used in the chromatography with nanospray ionization into the Thermo LTQ XL linear ion trap mass spectrometer. Preliminary method development include 1) steroid hormone detection from a 50 mg ww blubber sample and 2) cholic acid validation as a viable internal standard for quantitation. Standard curves for cortisol, testosterone, and progesterone quantitation were generated using cholic acid as a surrogate internal standard with the following concentrations: 0.05 nM, 0.1 nM, 0.5 nM, 1 nM, 5 nM, and 10 nM. Cholic acid concentration was held constant at 5 nM and concentrations were reported as peak area ratios. The use of surrogate internal standards is very useful (Kunze et.al 2015), and cholic acid was chosen due to its steroidal structure and lack of measurable accumulation in the blubber samples tested. The small sample mass (50 mg) required for this new methodology for blubber steroid hormone analysis is compatible with biopsy collection in western gray whales and allows for concomitant genetic and stable isotope analyses. Methodology optimization to increase the number of detectable steroid hormones is underway.

28 The dissipation and transport of the glyphosate additive polyoxyethylene tallow amine (POEA)

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The surfactant POEA is an ingredient in many glyphosate formulations--the most widely applied agricultural herbicide in the world. POEA is toxic to many aquatic organisms and is often the primary factor of the toxicity of glyphosate formulations. The fate of POEA applied to agricultural fields and the potential transport of POEA into aquatic systems are generally unexplored research topics. A multiyear study of an active row crop field in Indiana documented that POEA, glyphosate, and a degradation product of glyphosate (AMPA, aminomethylphosphonic acid) persist from year to year with some dissipation from the top 15 cm of the field but little migration into deeper soil. The detection of POEA, glyphosate, and AMPA in stream bed sediments from agricultural and urban watersheds in Georgia, Hawaii, Iowa, Mississippi, North Carolina, and South Carolina indicate the dissipation of these compounds from the application site leads to their transport into nearby surface waters. This is the first study to simultaneously analyze a primary formulation surfactant, the active ingredient, and the major degradate of glyphosate formulations in agricultural soils and corresponding stream bed sediments.

30 Separation and analysis of single walled carbon nanotubes in estuarine sediments using density gradient ultracentrifugation with NIRF and ICP-MS

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Sensitive and reliable quantification and characterization methods are needed to facilitate the ecological risk assessment of carbon nanotubes, including single-walled carbon nanotubes (SWCNT) in the aquatic environment. These analyses are particularly challenging because of the association of SWCNT with particulates (e.g. sediments) and the resulting complexity of samples. Near infrared fluorescence spectroscopy (NIRF) has been used to make sensitive measurements of SWCNT in marine sediments, but this technique is not sensitive to all SWCNT types. Metal catalysts are widely used in synthetic production of SWCNTs, leading to total metal content ranging from 5 - 30%. Therefore, by monitoring the metal types and metal:metal ratio along with monitoring elemental carboncontent, SWCNTs can, in principle be quantified in environmental samples using ICP-MS. Background metals from sediment present challenges to such analyses, however. Thus, we have applied density gradient ultracentrifuge (DGU) to isolate and separate SWCNT in sediment extracts prior to both NIRF and ICP-MS analysis. Several types of SWCNTs (arc discharge, CoMoCat, and HiPCO) were spiked and subsequently extracted from estuarine sediments. SWCNTs were separated into different bands after DGU, primarily into two distinct horizons (one showed near infrared fluorescence, while the other did not). Two techniques,near-infrared spectroscopy (NIRF) and ICP-MS, were applied for quantitation of SWCNTs in these bands. Results indicate excellent separation of SWCNT from interferences in sediments.We have also discovered an apparent disconnect between the metal catalyst particles and SWCNT during density gradient ultracentrifuge separation. It is clear that the SWCNT (within the NIRF band) is not physically associated with metal catalyst. This result was confirmed using single-particle ICP-MS. Although DGU separation seems to be an outstanding method for isolating SWCNT from aquatic sediment for analysis, ourcurrent findings indicate that metal fingerprints derived from residual catalyst may not be a good tracer for SWCNT occurrence and fate in marine sediments, as the associated metal catalyst particles in SWCNT preparations might be transported in different ways relative to the SWCNT.

31 Determining the Dissolution Rate of Nanomaterials in the Aquatic Environment by Ultra-Centrifugation

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The environmental fate and toxicity of nanomaterials are highly influenced by their dissolution kinetics (rate of solubility) and equilibrium solubility (amount of dissolved material). Solubility is material-dependent, but not as such an inherent property. In addition to the material properties, dissolution also depends on the water chemistry of the surrounding media (e.g. ionic strength, ligands, pH, organic matter and temperature). For other nanomaterials, the following parameters will also influence solubility: particle size, state of aggregation and particle coating. It is important to understand nanomaterial dissolution to be able to predict their fate and behavior in the environment. Also, information on particle dissolution is important in the interpretation of toxicological test results. The authors are currently leading the development of

Organization of Economic Cooperation and Development (OECD) standard guidance for determining the dissolution rate of metal nanoparticles. Here we propose a screening study testing up to 3 concentrations of nanomaterials and 3 pH levels for 24 hr in 5 mM sodium bicarbonate water to determine those particles which undergo rapid dissolution such that their ecotoxicity potential is practically indistinguishable from soluble forms. If only partial or no dissolution occurs, a full study is proposed to determine dissolution kinetics and incorporate specific conditions such as media composition, concentration, and pH level lasting up to 28 days. Phase separation methods were tested and we determined that ultra-centrifugation was preferable compared to ultrafiltration and dialysis due to the potential for adsorption to membranes/filtration media and the ability to reduce variability. We have found that polypropylene or high density polyethylene resulted in less adsorption compared to polycarbonate and glass. The results of these studies will be used to develop a draft protocol for dissolution ring tests in the development of a standardized guidance for dissolution rate and ultimately support the environmental hazard classification of metal nanoparticles.

32 Determination of lead in American woodcock (Scolopax minor) feathers and bone with temporal analysis and lead source determination

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Lead (Pb) concentrations are known to exceed biologically relevant thresholds in American woodcock (Scolopax minor). This may be contributing to their negative population trend. Woodcock are susceptible to Pb accumulation through ingesting earthworms, contaminated soil, and Pb shot. Although lead shot was banned for use on waterfowl, it is postulated to be the primary source of elevated Pb in American woodcock. Lead determination was performed using primary feathers (P2), secondary feathers (S14) and wing bones from juvenile woodcock. This allowed for a temporal study because the three sample types provide information on natal origins, migration routes and lifetime Pb accumulation, respectively. All samples were analyzed for total Pb concentration by using an inductively couple plasma – mass spectrometer. Isotope ratios of $^{206/207}$Pb and $^{208/207}$Pb were calculated to determine the sources of Pb woodcock are exposed to. Elevated Pb concentrations (> 4 ppm) were found in 19% of the feathers, with 24% having concentrations ≥ 20 ppm. Approximately 60% of the feathers with elevated Pb were P2 feathers, indicating Pb accumulation is slightly greater at woodcock natal origins. Isotope ratios in both the P2 and S14 feathers overlap with ratios from multiple environmental sources including coal emissions, gasoline, zinc smelting, and various types of Pb shot. The data collected confirm that woodcock are exposed to elevated Pb concentrations. While Pb shot is a possible source of Pb in woodcock, further research is needed to accurately determine the source of Pb in birds with elevated Pb concentrations.

Wildlife Ecotoxicology: From Food Chain Exposure to Population Effects

33 Thyroid Pathology in Deer Mice (Peromyscus maniculata) from a Reclaimed Mine Site on the Oil Sands

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Free living small mammals are sensitive, useful and available sentinels which can be studied to evaluate ecological and health risks from pollutants. Information from this small mammal-based investigation may provide insights at higher levels in the food web, and have implication ergarding sustainability of local ecosystems. The extraction of bitumen in areas of northeastern Alberta, Canada, has been associated with the release of complex mixtures of metals, metalloids, polycyclic aromatic compounds, and other compounds to the environment. In previous studies of northern rodents during the spring breeding season, we observed that deer mice (Peromyscus maniculata) from a reclaimed mine site had higher renal levels of cobalt (Co), selenium (Se), and thallium (Tl) compared to the deer mice from a reference site in the same region, but beyond the range affected by airborne deposition of most industry related contaminants. This increased exposure to metals plus other unmeasured contaminants was associated with poorer body condition, smaller testis mass, and increased oxidative stress which was determined through vitamin A and glutathione redox status in both the liver and testes. These findings stimulated further investigation into the health effects, specifically histopathological examination and hormone production of the thyroid glands. Information or reports of naturally occurring thyroid disease in wild animals in general, and in small mammals specifically, is extremely limited. We therefore investigated the structure and function of the thyroid glands and thyroid hormone levels of the deer mice captured on reclaimed and reference sites on and near mining areas on the Athabasca oil sands in Alberta. Mice from the reclaimed site had marked thyroidal changes including follicular cell proliferation and decreased colloid in the follicles compared to animals from the reference site. The abnormal follicular hyperplasia was significantly correlated with higher oxidative stress (p=0.02), as was decreased thyroidal colloid production (p=0.05). Thyroid hormones, both thyroxine and triiodothyronine, were higher in animals with greater oxidative stress (p=0.01) indicating increased metabolic demands from the contaminant related oxidative pressures. This work confirms the value of a combination of endocrinological, histologic and oxidative stress biomarkers for providing a sensitive and meaningful biological measure of the cost of contaminant exposure in native small mammals on the oil sands.

34 Influence of PAH exposure and diet on metabolomic responses of double crested cormorants

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Field based studies have the unenviable challenges typical of uncontrolled experiments; confounding factors, difficulty in establishing cause-effect relationships, and causal factors that were not considered during the experimental design. In this study, we investigated the putative differences in metabolomic responses of double crested cormorants from 3 colonies in relation to differences in airborne exposure to polycyclic aromatic hydrocarbons (PAHs), dietary exposure to POPs, and diet. Earlier research indicated that airborne exposure to PAHs increased the incidence of mutations in double-crested cormorants that were breeding in Hamilton Harbour relative to those from a Lake Erie reference site; further, alterations in gene expression in the putative p53 (tumor suppression gene) pathway were observed. We examined the metabolomic responses from cormorant chicks from each colony, using 3 tissues (blood, liver, lung). Over 200 metabolites, including acylcarnitines, amino acids, glycerophospholipids, hexose, sphingolipids, and biogenic amines, fatty acids, and bile acids, were measured by flow injection- or liquid chromatography-tandem mass spectrometry. Although all three tissues easily differentiated the three colonies, the majority of differences observed were between the Lake Erie colony and the other two Hamilton Harbour colonies. Based upon estimates of diet using regurgitates and stable isotopes (C,N), most of the differences in metabolomics responses appear to be due to diet, as opposed to either airborne PAH exposure, or dietary exposure to POPs. Metabolomics are a useful tool in determining responses of wildlife in field studies where there are multiple, sometimes confounding, stressors including contaminant exposure and ecological drivers.

35 Neurotoxic impacts of mercury on Arctic Barnacle goslings, raised in moderately contaminated terrestrial habitats in the Arctic

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Although the Arctic is remote, it is contaminated by mercury from different sources. Via long range transport, mercury is transported for from lower latitudes to the Arctic via the atmosphere. Additionally, local human activities may also result in contamination. For instance, mining activities have resulted in elevated levels of mercury in the surroundings of the mining areas. The focus of mercury related research in the Arctic has mainly been on marine ecosystems, which are regarded most at risk. Risks associated with mercury in terrestrial ecosystems have been less studied. In the current presentation we will present a pilot-study in which we herded Barnacle goslings in a contaminated mining site and a control site near Ny-Ålesund, Spitsbergen. Herding the goslings made it possible to perform a controlled field study, quantifying the accumulation of mercury in the goslings. Soil in the mining area contained significant higher concentrations of total mercury than the control site. This was also the case for the vegetation (approx. 2.2 times higher in the mining site than the control site). Each group of goslings grazed approximately 3-4 hours per day in their assigned area. At night and during bad weather conditions they were kept in pens. The goslings that grazed in the mine impacted area showed increased levels of total mercury in the liver in comparison to the control group ($p < 0.05$). Concentrations were relatively low, possibly due to growth dilution due to growing feathers. Nevertheless, dopamine receptor levels in the brains of the goslings were significantly related to the hepatic total mercury concentrations. Furthermore, the behaviour of the goslings from the mine-exposed group was significantly different than from the controls. The exposed goslings showed significantly more jumps during controlled observation periods. This study indicates that, although exposure of organisms to mercury in terrestrial Arctic ecosystems may be relatively low, it still results in neurotoxic effects and even effects on the organisms' behaviour. In case of barnacle goslings this may have grave consequences when e.g. interactions between parents and goslings are affected. This warrants for further studies, integrating mechanistic biochemical ecotoxicological observations with ecological relevant observations in behaviour and other fitness parameters.

36 Assessment of Lead and Pesticide Exposure in the Declining Population of Red-breasted Goose (Branta ruficollis) Wintering in Eastern Europe

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The red-breasted goose Branta ruficollis is a globally threatened species (IUCN Vulnerable) and the only European goose species currently in decline. Working on the wintering grounds on the Black Sea Coast, we address two potential causes of decline of this species for the first time: lead poisoning, and contamination from pesticides. We quantified the densities of spent Pb shot in three wetlands used by the geese in north-east Bulgaria, and analysed the Pb concentration in the faeces of red-breasted geese and the more abundant greater white-fronted geese Anser albifrons, using Al concentration as an indicator of soil ingestion. Pb shot densities in sediments were low, and we found no evidence for Pb shot ingestion in red-breasted geese. On the other hand, we found that the geese were feeding on wheat whose seeds were treated with four fungicides: thiram, tebuconazole, difenoconazole and fludioxonil. Using data on the daily food intake, we estimated the exposure levels of the geese to these fungicides, both by measuring the concentrations remaining on seeds and by estimating the amount used to coat the seeds at the time of sowing. We found that the estimated ingestion rates for both geese species exceeded the recognized hazardous doses for thiram, and to a lesser extent for tebuconazole, which indicates that some pesticides may be playing a previously overlooked role in the decline of red-breasted geese.

37 The catbird is the new chicken: high sensitivity to a dioxin-like compound in a wildlife species

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Avian toxic equivalency values used for risk assessments of dioxin-like compound (DLC) contaminated sites are based on chicken toxicity testing; however, to date, no wildlife species in North America has been verified to be as sensitive as the chicken. Recently, the Gray catbird (Dumetella carolinensis), a North American songbird, was predicted to be highly sensitive to DLCs based on genotyping of the aryl hydrocarbon receptor (AHR1) ligand binding domain. The objectives of this study were to test the sensitivity of the Gray catbird to the embryotoxic effects of a dioxin-like PCB (PCB-126), and to compare the induction of hepatic CYP1A4/5 mRNA expression between the catbird and the chicken following PCB-126 exposure. Eggs were collected from the South Okanagan Wildlife Management area in British Columbia, Canada, injected with 0.5 µl/g of the vehicle control (DMSO) or one of four PCB-126 concentrations (0.71, 5.36, 11.42 and 39.98 ng/g egg), and artificially incubated to hatch. We also injected chicken eggs with DMSO, 0.01, 0.1, 0.5, or 1 ng PCB-126/g egg, and incubated until hatch to assess induction of CYP1A4/5 mRNA expression by PCB-126; this had not been previously assessed for chickens. There was a dose-dependent increase in catbird embryo mortality with a calculated LD50 of 1.35 ng PCB-126/g egg (95% CI = 0.34 to 2.54 ng/g), which falls within the range of published LD50 values for the chicken (0.4 to 2.3 ng/g egg). There was significant induction of both CYP1A4 and CYP1A5 mRNA in catbird hatchlings from the 0.71 ng PCB-126/g egg dose group. Similarly, in chickens there was significant induction of CYP1A4 and CYP1A5 starting at 0.5 ng PCB-126/g egg. Catbird CYP1A4 induction was 257-fold at 0.7 ng/g and 486-fold at 5.36 ng/g, and CYP1A5 induction was 55-fold at 0.71 ng/g and 87-fold at 5.36 ng/g. In the chicken at 1ng/g, CYP1A4 induction was 106-fold, and CYP1A5 induction was 52-fold. For both catbirds and chickens, CYP1A4 was much more induced than CYP1A5, which is typical of highly sensitive species. The results of this study demonstrate that the gray catbird is at least as sensitive as the chicken to DLCs, based on both embryotoxicity and CYP1A4/5 mRNA induction. This is the first instance of a North American wildlife species being confirmed to be highly sensitive to DLCs.

38 Continuing Immunological and Reproductive Impairments in Great Lakes Colonial Waterbirds Breeding in Contaminated Sites in Michigan during 2010-16

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This study assessed effects of contaminants, primarily PCBs and PCDDs, on immune function and reproduction in fish-eating birds in the Saginaw Bay and Raisin River Areas of Concern (AOCs) and Grand Traverse Bay during 2010-16 under the Great Lakes Restoration Initiative-Fish and Wildlife Service AOC program. Saginaw Bay sites included two herring gulls 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 in Grand Traverse

Bay on Bellow Island. Reference sites were in the lower St. Mary's River (gulls on Pipe Island Twins and terns on Two Tree Island) and on Chantry Island, Lake Huron (herons). Gull nests were marked during egg-laying, and viability was assessed during late incubation using an embryonic viability detector sensitive to heartbeat and movement. Embryonic nonviability in herring gulls in the Saginaw Bay and River Raisin AOCs (7.0% for the CDF, 7.0% for L. Charity, and 8.7% for Monroe) was higher than at the reference site (3.3%). Infertility was the primary cause of nonviability at the reference site. Both elevated infertility and embryonic mortality contributed to nonviability in AOCs. Two gull chicks at Monroe and one embryo on the CDF had crossed bills, a deformity associated with PCBs and PCDDs. In the Saginaw Bay AOC chick productivity was substantially below reference values in 3/5 years for Caspian terns on the CDF (with complete reproductive failure in 2015), 2/3 years for Caspian terns on Charity Reef and L. Charity Island, 1/6 years for herring gulls on the CDF, and 1/5 years for herring gulls on L. Charity Island. In the River Raisin AOC, productivity was very poor in 3/6 years, with complete reproductive failure during 2010. In herring gull chicks the mean phytohemagglutinin (PHA) skin response for T-cell mediated immunity was suppressed 53-57% at both AOCs and 50% in Grand Traverse Bay. This response was suppressed 46-50% in Caspian terns and 39% in black-crowned night herons in the Saginaw Bay AOC. Mean antibody responses were at least two-fold lower in herring gull chicks at both AOCs and in Grand Traverse Bay. Ongoing immunological and reproductive impairments at these contaminated sites are consistent with the effects of persistent pollutants, such as PCBs and PCDDs.

39 Environmental contaminants associated with bioindicator response in tree swallows nesting in US and Binational Great Lakes Areas of Concern

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From 2010 to 2014 tree swallow (Tachycineta bicolor) nestlings were collected from 69 sites in the Great Lakes basin including multiple sites within 27 Areas of Concern (AOCs) and nine nearby non-AOC sites. Polychlorinated biphenyl (PCB) and polybrominated diphenyl ether (PBDE) concentrations were measured in nestling carcasses and stomach contents. Polycyclic aromatic hydrocarbon (PAH) concentrations were measured in nestling stomach contents and perfluorinated compounds (PFCs) were measured in plasma. EROD activity and oxidative stress (8 measures) were measured in liver tissue. Chromosomal damage (DNA CV) was measured in red blood cells. Significant differences among sites and among AOCs were noted for all of the bioindicators. To identify the relative importance of contaminants to the various bioindicators, redundancy analysis was conducted. A model based on individual nestling values for PCBs, PCB TEQs, PFCs, PFOS and PBDEs (N = 230) indicated that the amount of variability within the bioindicators was significantly explained by PCBs (P=0.003) and PBDEs (P=0.025). Among the bioindicators, as EROD activity increased and the TBARS response decreased, the concentrations of PCBs and PBDEs increased. DNA CV and 7 other measures of oxidative stress were not associated with the contaminants measured. A second model based on dietary site means for alkylated PAHs (aPAHs), parent PAHs (pPAHs), PBDEs, and PCBs (N= 55) was significant. However, none of the contaminants predicted a significant amount of the variability. Concentrations of pPAHs did have the lowest significance value (P = 0.14) among the contaminants measured and sample size may have contributed to the lack of significance. A third model based on individual sibling egg values, which included measures of PBDEs, PCBs, PCB TEQs, dioxins/furans, dioxin/furan TEQs, and selected pesticides (8 total) (N = 220) was significant and indicated that only dioxin/furan TEQs explained a significant (P = 0.02) amount of the variability in the data. As EROD activity increased and the TBARS response decreased, the concentrations of dioxin/furan TEQs increased.

40 Pharmaceutical and personal care product exposure in ospreys in Chesapeake and Delaware Bays: largely a clean bill of health, is this a surprise?

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Concerns over potential exposure and effects of pharmaceuticals and personal care products (PPCPs) in non-target wildlife have grown as a result of population level effects in fish (e.g., synthetic hormones) and Asian vultures (i.e., diclofenac). In Chesapeake (CB) and Delaware Bays (DB), wildlife were adversely affected by some environmental contaminants during the 20th century. The piscivorous osprey (Pandion haliaetus) has been used as a sentinel of estuarine health for decades. Between 2011 and 2015, osprey eggs (n= 65 for CB and 27 for DB) and nestling plasma (n= 69 for CB and 29 for DB) were collected and analyzed by MS for PPCPs. Methoxytriclosan was detected in 17 of 65 CB eggs (0.4-7.4 ng/g egg ww) from CB, but levels of this degradant are orders of magnitude below concentrations associated with triclosan toxicity in higher vertebrates. The antihypertensive diltiazem was detected above method detection limits (MDL) in all plasma samples from CB (0.56-8.63 ng/mL), while 22 other analytes were not. In DB only 2 of 20 analytes were detected above MDL (analgesic acetaminophen in 22 of 29 samples, 1.42-3.95 ng/mL and the NSAID diclofenac, 2 of 29 samples, 2.33, 3.73 ng/mL). Analysis of water samples (analytes detected: CB = 18 of 23, d DB = 7 of 20) and osprey forage fish (CB =8 of 23, DB = 8 of 20) indicated exposure to multiple pharmaceuticals that did not bioaccumulate in ospreys. While it is of interest that we observed greater concentrations ($p< 0.05$) in areas with industry and urbanization, it is unlikely statistical significance extends to ecological risk. In all cases, osprey plasma concentrations were 2-3 orders of magnitude below human therapeutic plasma concentrations (diltiazem = 400 ng/mL, acetaminophen = 5000 ng/mL and diclofenac =500 ng/mL). There was no evidence that exposure to PPCPs correlated with effects at the molecular (oxidative DNA damage) through population levels (productivity adequate to sustain the populations in both estuaries). A literature review of wildlife and PPCPs revealed our negative data are perhaps part of a trend indicating PPCP exposure of wildlife is orders of magnitude below effects levels in mammals. Beyond a few instances of population level effects (fish and Gyps vultures), existing data and read-across approaches suggest that adverse effects in wildlife should only result in exposed species with vastly different metabolic pathways or receptive sensitivities than found in laboratory mammals and humans.

Assessing Risks of Pesticides to Federally Listed (Threatened and Endangered) Species at a National Level – Part 1

41 Process and criteria for efficient assessment of pesticide risk to endangered species

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Assessment of pesticide risk to endangered and threatened (listed) species on a national scale is a conceptual and logistical challenge. The assessment process must be able to discriminate degrees of risk efficiently across a large number of listed species so that resources can focus on species for which pesticide use has the greatest likelihood of harming individuals and populations. Because risk can never be entirely precluded, explicit protection goals and objective scientific criteria are a critical foundation for regulatory decisions. The risk criteria should address both the magnitude and probability of potential effects; small effects can be tolerated with greater frequency than large effects. The process used in the recent EPA Biological Evaluations (BEs) of 3 organophosphorus insecticides relied extensively on estimated exposure concentrations (EECs) derived using screening-level exposure models, which were taken at face value with virtually no consideration of the probability of those concentrations occurring within the habitat of any given species. Moreover, the BEs failed to distinguish species at high risk from those at lower risk; instead, they concluded that nearly all listed species (including plants) were at risk from these insecticides and required evaluation by the US Fish and Wildlife Service and National Marine Fisheries Service. The process for assessing risk to listed species can be much more efficient if the first step includes a standard screening-level ecological risk assessment (comparable to the process that has been used for decades to make pesticide registration decisions) to eliminate taxonomic groups of low concern from further investigation. Decisions on risk to individual species should then be made in the context of objective criteria using a weight of evidence analysis that effectively categorizes species according to the level of risk.

42 A Risk Assessment Process for Establishing Negligible Risk Earlier in National-Scale Endangered Species Assessments

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The stepwise endangered species risk assessment process detailed in the Interim Approach combined with more recent approaches communicated by the Federal Agencies (hereafter referred to as “Agency Methods”) in the draft Organophosphate Biological Opinions has yielded useful insights regarding the utility and practical limitations of these methods for risk assessment screening purposes. Agency Methods, while conservative by design, do not clearly incorporate opportunities to take advantage of readily-available information and simple strategies that can optimize the screening ability of the process while maintaining adequate conservatism desired for listed species protection. When strictly following Agency Methods, the number of listed species and habitats where risk is presumed, and therefore require a jeopardy evaluation, is excessive but the level of species relevance and best use of data lacking. As a result, the effectiveness of a tiered assessment process is greatly reduced. Further, the boundary between approaches and refinements used in Step 1 (“May Affect/No Effect”) and Step 2 (“Likely/Not Likely to Adversely Affect) can become unclear and even irrelevant due to the iterative nature of risk assessment. Regardless of the formal step outlined, there is a practical continuum of refinements and approaches that can be applied to effects and exposure analyses to establish negligible risk earlier in the risk assessment. The framework and decision criteria used within the framework should not be such that “Likely to Adversely Affect” decisions are commonly reached due to arbitrary limitations without fully utilizing available species habitat and biological data, species relevant exposure estimates, and surrogate ecotoxicity data. To that end, a systematic process for efficiently and effectively determining negligible risk for species and their habitats early in an assessment is presented. The approach is based on a set of fundamental “best-practices” developed from experience in conducting listed species assessments.

43 Interim Methods Used in the Biological Evaluations to Estimate Risk to Individuals of Threatened and Endangered Species from the Use of Pesticides

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The National Marine Fisheries Service (NMFS), U.S. Department of Agriculture (USDA), U.S. Environmental Protection Agency (USEPA), and U.S. Fish and Wildlife Service (USFWS) have developed interim approaches to determine the potential impact to individuals of threatened and endangered species from the use of pesticides at the national level. In April of 2016 draft Biological Evaluations (BEs) using the interim approach for three pilot chemicals (chlorpyrifos, diazinon, and malathion) were released for public comment. Additionally, a workshop was held in the summer of 2016 to allow for additional input from the public and stakeholders on specific technical issues (e.g., exposure in flowing waterbodies, geospatial and process refinements to the interim method, and weight of evidence). This presentation provides an update on the BEs based on the continuing work of NMFS, USDA, USEPA, USFWS; public comments; and the stakeholder workshop.

44 Assessing Risks to Plants Under the Endangered Species Act Process

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The U.S. Fish and Wildlife Service, National Marine Fisheries Service, and U.S. Environmental Protection Agency have been developing an approach for evaluating risk from pesticides to federally listed threatened and endangered plants and their designated critical habitats. Currently there are approximately 900 threatened and endangered plants and 450 critical habitat areas associated with these plants that encompass a variety of taxonomic groups (flowering plants, lichens, ferns, other allies), life history traits, and habitat types. A unique challenge to developing the methods for this diverse group of organisms has been utilizing existing toxicity data on insecticides, where the mechanism of action in plants is poorly understood, and there are few data available. An additional challenge for these plant reviews was to consider not just direct effects to the plants themselves but also indirect effects from reliance on pollinator or seed dispersal mechanisms which may also be unknown or poorly understood for many species. An overview of the current methods and specific challenges to these assessments such as use of plant toxicity data, assumptions related to overlap of species ranges with pesticide use, and the identification of pollinators, will be discussed.

45 Estimating the proportion of a bird population exposed to a single pesticide

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The United States Environmental Protection Agency, in collaboration with the US Fish and Wildlife Service and National Marine Fisheries Service recently drafted national level biological evaluations for endangered and threatened (listed) species potentially exposed to chlorpyrifos, diazinon and malathion. In these draft biological evaluations, it was concluded that these chemicals have the potential to cause mortality or sublethal effects to individuals of most listed species. The risk and confidence in these conclusions are high for listed birds given the high acute toxicity of chlorpyrifos and diazinon to these species, known reproductive effects of malathion, and the likelihood that exposures in treated fields and adjacent areas will be of the magnitude where toxicity is observed for all three pesticides. The draft biological evaluation was conducted to determine if exposure to these chemicals was likely to cause an adverse effect to at least one individual of a listed species over the course of the 15 year action. In order to move to the next phase of the pesticide consultation process, it is necessary to derive an estimate of the number of individuals within the population that are likely to be exposed to the pesticide each year. We examine variable exposure scenarios by considering pesticide use as described on labels as well as potential factors that may impact the spatial extent of use over time, such as rotation of crops, proportion of crop historically treated, and application timing. This range of exposure scenarios is overlaid with biological information for species that influence the geographic and temporal distribution within its range. Information such as preferred habitat types, timing of breeding, migration, and feeding habits of different life stages are incorporated into the analysis to better predict the likely number of listed individuals that could encounter pesticide exposure(s). A case study with the least bell's vireo (Vireo bellii pusillus) and applications of diazinon is presented.

46 Use of population modeling in national Endangered Species Act Consultations with Pesticides

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A Biological Opinion (BO) is a statutory requirement under the Endangered Species Act (ESA) when a Likely to Adversely Affect determination triggers a consultation by the U.S. Fish and Wildlife Service or the National Marine Fisheries Service (Services) to assess potential impacts on a federally-listed endangered or threatened species. The purpose of a BO is for the Services to determine whether a federally proposed action jeopardizes the continued existence of a listed species or adversely modifies (or destroys) designated critical habitat. Because the focus of the BO is at the population and species scales, population models can be useful in assessing potential outcomes that result from an action. A National Academy of Sciences Panel Report discussed and supported the use of population models as a component of ecological risk assessments in the context of BOs regarding the U.S. Environmental Protection Agency's pesticide registration program. This presentation will describe the role population models may play in pesticide BOs for assessing risks to populations of listed species. We will also discuss population model structures and capabilities, data requirements (representing species biology and life history as well as quantitative links between and pesticide exposures and effects), uncertainties, and limitations of model use. Where applicable, population models can inform specific lines of evidence included in a BO. Such models include those applied in Pacific salmonid BOs on several current-use pesticides as well as others being assessed.

47 Species-specific refined endangered species risk assessment for static aquatic habitats: Part 1, exposure

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The current USEPA approach to static aquatic exposure modeling at Step 2 of their interim process for national endangered species assessments uses simplified assumptions about water body characteristics, the surrounding landscape, and agronomic practices. The USEPA approach considers generalized aquatic habitat dimensions, a homogeneously cropped watershed with 100% cropped area, up to two weather time series, one soil profile, and one pesticide application date pattern per crop group and HUC2. The same predictions of pesticide concentrations derived from these generalized assumptions are assigned to all of the species within a given HUC2 regardless of the location of that species range with respect to different agricultural crops, weather, and soils. The refined exposure modeling approach presented here for malathion focuses on quantitatively accounting for observed variability in environmental and agronomic factors that impact the potential pesticide exposures to endangered species. For each species, probability distributions of application timing, weather, soil and slope conditions, and crop configurations around ponds are sampled to generate on the order of 1000 30-year Pesticide Root Zone Model (PRZM)/Variable Volume Water Model (VVWM) realizations. Each realization is composed of multiple PRZM simulations, with the number of simulations being determined by the cropping pattern complexity around a given pond. The refined approach led to more realistic predictions of pesticide concentrations for a wide range of environmental conditions in unique species ranges. Assuming 100% treated area, concentrations for many species were two to three orders of magnitude lower than suggested by the USEPA Step 2 analysis. Accounting for actual treated area based on eight years of recent malathion use data resulted in more realistic concentrations. This methodology is readily reproducible and extensible to assess aquatic species across the United States. Risk results corresponding to the exposure concentrations generated by the probabilistic approach are the topic of a companion presentation.

48 From Biological Evaluation to Biological Opinion: What to expect next with the National Pesticide Consultations

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Current national pesticide consultations are transitioning from evaluating effects to individuals of threatened and endangered species and individual features of their critical habitats (assessed in Biological Evaluations), to evaluating effects on populations, species, and their critical habitat designations (assessed in Biological Opinions). In both documents, ecological risk assessments are used as the organizing framework to determine whether and to what degree the registration of a pesticide as described by current labels affect listed species and their habitats. In April 2016, draft Biological Evaluations for chlorpyrifos, diazinon, and malathion were released for public comment. Each concluded that hundreds of threatened and endangered species and their critical habitats were "likely to be adversely affected", thus triggering the next step of the Endangered Species Act consultations, a Biological Opinion for each of the pesticides. The Biological Opinions will also consider the adverse effects in the context of the environmental baseline, status of the species and their critical habitats, and cumulative effects to determine whether species would be jeopardized and/or their critical habitats would be adversely modified by registration of each pesticide. Since completing the draft Biological Evaluations, staff from the U.S. Environmental Protection Agency, U.S. Fish and Wildlife Service, and National Oceanic Atmospheric Administration's National Marine Fisheries Service (Agencies) began evaluating risk assessment methodologies for determining whether species would be jeopardized and whether designated critical habitat would be adversely modified. An update on the Agencies' progress will be provided that includes conceptual frameworks, key concepts, methodologies, and weight of evidence approaches.

History and Role of SETAC in the Advancements in Environmental Chemistry and Aquatic Toxicology

49 Fire on the water and other stories: The genesis of the science of ecotoxicology

B.A. Stubblefield, Oregon State Univ / Environmental and Molecular Toxicology; T.J. Norberg-King, USEPA / NHEERL/Mid-Continent Ecology Division

In the 1950's, 1960's, and 1970's, a variety of social, economic, and environmental factors in the US led to public concerns regarding environmental quality and public safety. The need to address questions regarding the environmental effects resulting from man's activities and the development of methods and tools with which to address these questions became important during this period. Responses to these concerns led to a number of activities including: the creation of the EPA and the development and implementation of a variety of environmental regulations (e.g., Clean Water Act, Toxic Substance Control Act, Clean Air Act); the development of training programs at universities for educating toxicologists and environmental chemists; the conduct of a great deal of research in academic, industry and government laboratories aimed at the evaluation of environmental contaminants, development of data to satisfy regulatory requirements, and the derivation of criteria/standards; and actions associated with evaluation and clean-up of contaminated sites. Historical perspectives and driving factors will be discussed as they relate to the current state of the science.

50 SETAC's role in promoting environmental chemistry

D. Mackay, Trent Univ / Chemistry

It may not be fully appreciated that SETAC has played a major role in the evolution of the chemistry of contaminants, especially organic contaminants. That evolution continues today and aspects of this evolution are described from a personal perspective. The American Chemical Society (ACS) sponsored a 1964 report "Cleaning our Environment" the Chemical Basis for Action that documented those issues and made 72 recommendations for scientific action. It did not cite or mention Rachel Carson's earlier "Silent Spring" that had generated considerable hostility from the chemical industry! As clean up progressed and gross examples of pollution were successfully mitigated, concern focused more on lower concentration of contaminants and more subtle toxic effects. Clearly there was a need for closer collaboration between chemists, ecologists and toxicologists but there were limited opportunities for professional scientific dialogue. The formation of SETAC in 1979 prompted by professionals from the chemical industry and wildlife toxicologists changed all that. Especially enlightened were a group from Dow Chemical Co. (notably Gene Kenaga) who realized that there was a need to better understand quantitatively and thus manage chemicals introduced to the environment. Fundamental to the proposed structure of SETAC was required and enlightened and equal involvement of professionals from government, industry (including consultants and National Laboratories) and academics and especially their students. The SETAC recipe has proved highly successful professionally to environmental chemists by providing a common venue for meeting and publishing scientific findings. It has expanded to the Global professional society that we enjoy today. The founders and the volunteers who supported their vision were truly visionary and have had a major impact on and understanding of environmental chemistry and its linkages to toxicology.

51 SETAC's role in promoting environmental chemistry through Quantitative Structure Activity/Property Relationships. A – Correlations

P.H. Howard, SRC, Inc.

As noted by the two previous talks by Don Mackay, SETAC has played a major role in the evolution of the chemistry of contaminants, especially organic contaminants. That evolution continues today. In these two presentations, the major role of SETAC in developing correlations between molecular structure and properties will be reviewed. As noted in a brief review letter to the editor in the 1985 issue of ET&C by Robert Lipnick, researchers first started noticing a correlation between physical properties of a chemical and toxicity in the 1890's: in 1893, Charles Richet found correlation between water sol. and toxicity to fish for 7 organic chemicals; in the late 1890's Overton and Meyer found an increase in oil/water partition correlated to an increasing toxicity in a variety of organisms. By the mid 1960's Corwin Hansch and collaborators proposed QSARs using octanol/water partition coefficient (Kow) using linear free energy relationships (LFERs); the latter approach dates back to the mid 1930's. Right around the formation of SETAC, researchers such as Veith and Konemann were finding correlationships between Kow and fish bioconcentration and toxicity. About the same time, it was apparent to the USEPA that the Toxic Substance Control Act (TSCA) would require assessments of tens of thousands of new and present commercial chemicals that had very little physical/chemical or toxicity information. As noted by Lipnick in his letter to the editor, so many QSAR papers were submitted to the 4th annual meeting of SETAC, that another session had to be added and these sessions continued. Because of the focus of SETAC, QSARs in SETAC focused on physical/chemical properties, environmental fate, and ecological toxicity. Valuable collections of the correlations were published such as one by Gene Kenaga, the first SETAC president, on BCF and soil sorption – Ecotox Environ Safety 4: 26-38, 1980. Another was an extensive book by Warren Lyman and David Rosenblatt with support from U.S. Army – Handbook of chemical property estimation methods 1982, reprinted in 1990. That book reviewed many of the correlations between structure and properties – MP, BP, VP, Henry's Law constant, water solubility, Kow, BCF, soil adsorption, biodegradation, photolysis, hydrolysis, volatilization from water, etc. An update of that book was published in 2000 by a variety of authors with Don Mackay and Bob Boethling. These correlations will be reviewed in chronological order.

52 Water Quality Criteria: The history and development of a regulatory tool to protect aquatic systems

B.A. Stubblefield, Oregon State Univ / Environmental and Molecular Toxicology; R.J. Erickson, USEPA / ORD NHEERL Mid Continent Ecology Division

Central to most regulatory schemes aimed at the control of waterborne contaminants for the protection of aquatic life in fresh and marine waters is the development and enforcement of numerical chemical limits. These values are designed with the intent to be protective of most, or all, organisms in aquatic environments. In the US initial efforts aimed at the development of water quality criteria/standards can be traced initially to the California State Water Quality Control Board in 1952 and later to the 1963 McKee and Wolf reference from that same organization. Subsequently the USEPA, or its predecessor organization, published it's "rainbow" of criteria books starting in 1968 with the "Green Book", followed by the 1973 "Blue Book", the 1976 "Red Book" and finally the 1986 "Gold Book." The procedures used to derive these values have evolved over the years but generally rely on data from acute and chronic laboratory-based toxicity tests developed with a range of representative surrogate species. These data are subsequently evaluated using defined statistical procedures to derive "protective" numerical values. The history of the development of these procedures will be discussed.

53 A Retrospective on the Adoption and Application of Whole Effluent Testing in the US

T.J. Norberg-King, USEPA / NHEERL/Mid-Continent Ecology Division

The Clean Water Act (CWA) establishes the structure for regulating discharges of pollutants into the waters of the United States and regulating quality standards for surface waters. The CWA Act was significantly reorganized and amended in 1972 and gave EPA the authority to implement pollution control programs such as setting wastewater standards for the industry and water quality standards for contaminants in surface waters. However, the approach of applying water-quality criteria to control the release of chemicals into water bodies could not cover all potentially toxic pollutants in an effluent nor did it predict biological effects in the receiving waters. Increased production, use and ultimately, environmental releases of synthetic industrial chemicals and a growing public awareness of environmental issues during this time made the application of aquatic toxicity analyses to discharges a natural step. As the CWA made it unlawful to discharge any pollutant from a point source into navigable waters unless a permit was obtained through the National Pollutant Discharge Elimination System (NPDES) permit program; and in 1984, EPA issued a national policy, Policy for the Development of Water Quality-Based Permit Limitations for Toxic Pollutants, that proposed the use of toxicity data to assess and control the discharge of toxic substances to surface waters. Use of acute toxicity tests for effluents testing became routine as the tests provide a more direct estimate of the safe concentration of effluents in receiving waters. New methods to estimate chronic toxicity were developed for effluent testing and validated in field studies and interlaboratory studies. During this time, EPA introduced new test methods with new species, such as the 3-brood Ceriodaphnia dubia test along with abbreviated early life stage tests for other species (fathead minnow, mysid shrimp, inland silverside, sheepshead minnow and more). In this presentation, I will touch on the highlights of the development of the use of whole effluent toxicity and field validation of the method to support the NPDES program. This presentation will cover the highlights of the method development and application to discharges of the US, along with highlighting the vetting of the science through SETAC which in turn supported environmental management decisions. Disclaimer: This presentation does not necessarily reflect the views or the policies of the USEPA.

54 SETAC as Matchmaker Between Chemists and Toxicologists: Two Personal Examples

D. Mackay, Trent Univ / Chemistry

There was a growing realization in the 1970s that organic contaminants did not respect the convenient boundaries between the traditional physical and regulatory media of air, water, soils and sediments. Images from the Moon landings clearly showed the need to address the whole planetary system of linked media, not just its detailed contaminated parts. The concept of chemical fate in connected "unit worlds" employing the fugacity concept was warmly supported within SETAC, especially by Dow Chemical Co. resulting in the emergence of multimedia fate and transport models that now have the potential to treat chemical behavior at scales ranging from small ponds to regional, continental and even global scales. There is increasing scientific and regulatory acceptance that such models can provide a sound basis for predicting and managing chemical fate, especially when coupled with emerging information technologies for data storage, analysis and open dissemination. Second is a brief review of bioconcentration that was originally a puzzling phenomenon noted by Rachel Carson and others. This proved to be a beautiful example of how SETAC brought together scientists from diverse backgrounds in chemistry, biology and ecology to establish our present understanding of bioaccumulation, biomagnification and trophic magnification in a variety of ecosystems and species.

55 SETAC's role in promoting environ. chem. through Quantitative Structure Activity/Property Relationships. B – Computer assisted calculations

P.H. Howard, SRC, Inc.

Initially in the late 1970's and early 1980's, correlations were made between Kow and BCF, fish toxicity, or fate properties. This required that you have a measured Kow value or do a hand calculation to estimate the value. Examples of some of these early hand calculations will be given. One of the earliest computer programs to estimate a property (in this case, Kow) from the chemical structure was CLOGP from Leo and Hansch at Pomona College. This program would take the chemical structure entered as a Simplified Molecular Input Line Entry System (SMILES) string and the computer would add or subtract the coefficients of the atom or functional group fragments to give the estimate. Different investigators used different chemical descriptors and correlated then to numerous properties. For example, Kier and Hall used molecular connectivity indices (MCIs); Syracuse Research Corp/EPA used hand selection of atom/functional group coefficients while other groups used computer programs to select atom/fragments; many of the programs in the OECD Toolbox use degradation pathway rules; and other researchers use molecular orbital calculations (the latter could take long periods of time until computers got faster). Development of the correlation between the chemical descriptor and the activity/property/degradation values used a variety of statistical approaches (linear and non-linear regression, neural networks, partial least squares, etc. The number of end points that could be estimated grew as papers were presented at the SETAC annual meeting and published in ET&C. A brief listing of these programs and endpoints with a little history of when they were developed and their methodology will be presented – these include Daylight software, CHEMEST, ECOSAR, SPARC, EPISuite,™ ACD, ChemAxon, ChemSpider, and the OECD Toolbox. Future needs for QSARs will be discussed.

56 A Retrospective on the Development and Use of Toxicity Identification Evaluations for Whole Effluent Toxicity (WET) Applications

T.J. Norberg-King, USEPA / NHEERL/Mid-Continent Ecology Division

Whole effluent toxicity (WET) testing is a major component of USEPA's Integrated approach to water-quality–based toxics control and a complement to aquatic-life–chemical-specific criteria and biological assessments. Regulatory authorities have been encouraged to use all of these tools to prevent the discharge of toxic pollutants to protect the nation's waterways. To determine what causes the toxicity observed in the WET test, the EPA-Duluth Laboratory began developing various laboratory methods in the early 1980s. We used the WET test(s) to identify the cause(s) of toxicity in effluents under the complex effluent toxicity testing program and applied developing TIE techniques to effluents tested during field studies and in the laboratory. The general procedures were described in freshwater TIE methods manuals for wastewaters and receiving waters. At the same time, USEPA-ORD established a technology transfer center for the water-quality–based permits, technical assistance, and TIEs at Duluth. During this period, numerous refinements of the methods and new approaches for the TIE methods were developed. Many of the chemicals identified through TIEs were not covered under the chemical-specific limits included in NPDES permits or were not expected to cause toxicity at concentrations present. EPA also developed TIE techniques for marine waters using the freshwater TIE approach as a model yet incorporated guidance on the interaction of seawater with the TIE manipulations. These methods have been applied, and further advanced through numerous applications by industry and consulting laboratories. This presentation will cover the highlights of the method development along with highlighting the vetting of the science through SETAC, which in turn supported environmental management decisions. Disclaimer: This presentation does not necessarily reflect the views or the policies of the USEPA.

Deepwater Horizon Oil Spill – The Discoveries and Outreach

57 Sharing the science behind the spill: The partnership of the Gulf of Mexico Sea Grant programs & the Gulf of Mexico Research Initiative (GoMRI)

E.S. Maung-Douglass, Louisiana Sea Grant – LSU / Louisiana Sea Grant; L. Graham, Mississippi-Alabama Sea Grant Consortium; C. Hale, Texas Sea Grant; S. Sempier, Mississippi-Alabama Sea Grant Consortium; M. Wilson, UF/IFAS Florida Sea Grant Extension

Deepwater Horizon oil spill occurred in 2010 off the coast of Louisiana (USA) and continues to be the largest accidental release of oil on record. While scientists from various sectors (e.g., research initiatives, government, environmental consulting) are making major advances in understanding the implications of the incident, effectively communicating those scientific findings continues to be challenging. Two years ago, the Gulf of Mexico Sea Grant programs partnered with the Gulf of Mexico Research Initiative (GoMRI). The resulting outreach program extends oil spill science to target audiences who could utilize oil spill science in decision-making and/or who depend on the Gulf for their livelihood. These target audiences include, but are not limited to, emergency responders, natural resource managers, elected officials, fishermen, and tourism-dependent businesses. Since 2014, our program has met with more than 1000 people within our target audiences from communities across the Gulf of Mexico. Based on our assessment of their needs, we work collaboratively with scientists from all sectors to help us create scientifically accurate extension publications and informational seminars that are a synthesis of published literature from a wide range of areas (e.g., seafood safety, impacts to aquatic life, fate of oil and dispersants). As our program enters its second phase in Fall 2016, we will share approaches used and lessons learned during Phase I, including the need to connect audiences that have not traditionally worked to improve communication during future spills, and unexpected challenges encountered while working with our target audiences and disseminating the science of the spill. More depth on deliverables, program structure and evaluation, as well a peek at what is to come in Phase II of the program will be shared.

58 Polycyclic aromatic hydrocarbons in white shrimp following the Deepwater Horizon accident: community-based science from southeast Louisiana

J. Wickliffe, Tulane Univ; D. Nguyen, Mary Queen of Vietnam Community Development Corporation; M. Wilson, B. Simon, J. Howard, Tulane Univ; S. Frickel, Brown Univ

The Deepwater Horizon Oil Spill had a profound impact on communities living and working along the US Gulf Coast. One could easily argue that the impacts were felt well beyond the US in myriad ways including research, science, policy, and how those are interconnected. Among those most deeply impacted were our fishing and seafood harvesting communities. Generally speaking, research intensive academic institutions and surrounding seafood-reliant communities do not have "shovel-ready" working relationships. Specifically, those that would allow them to jointly respond to environmental health issues during and immediately following a coastal disaster. This was not the case as we report our research results here. We worked as a team (academic researchers and shrimpers) to develop a plan to address paramount concerns regarding the quality of white shrimp that could or would be harvested in 2010 immediately following the capping of the Macondo well. This was done in a culturally appropriate and transparent manner to produce high quality scientific results (i.e. publishable) and meaningful information to our local seafood-reliant communities. In short, we developed a sampling strategy that addressed community- and fisherfolk-specific concerns and used analytical chemistry to determine the presence and quantity of 81 polycyclic aromatic hydrocarbons (both pyro- and petro-genics) in white shrimp from distinct sites in southeast Louisiana. Very few PAHs were detected in white shrimp collected in November 2010, consistent with the findings of other researchers and health agencies. Survey data regarding consumption behaviors specific to heavy consumers (Vietnamese-American shrimpers and their local community) were used to develop relevant and comprehensive probabilistic risk assessments. No unacceptable health risks were found even among the heaviest consumers with respect to PAHs with known toxicities. Together, the results of this community-based research project were effectively communicated back, including translations into Vietnamese, to this and other communities. In addition, research results have also been published in peer-reviewed scientific journals. While working with communities as part of a research effort requires patience, time, and energy, our work clearly demonstrates that it can produce meaningful results for all stakeholders that are involved.

59 Risk Messaging and Public Perception Among Gulf Coast Residents After the Deepwater Horizon Oil Spill

A.S. Kane, Univ of Florida / Dept of Environmental and Global Health

Developing and delivering meaningful risk communications for vulnerable or impacted communities presents dynamic and often multifaceted challenges for researchers. In response to Gulf coast community concerns regarding safety of inshore-harvested seafood after the DWH oil spill, we conducted analytical toxicology for oil spill-related contamination in fish, shrimp, blue crab and oyster, and discerned seafood consumption rates for Gulf coast residents relative to these same seafood categories. This presentation describes approaches taken to provide risk messaging and hazard communications back to participating communities. Many study participants were seafood workers who depend on ecosystem services for their livelihood, and personal identity relative to their multigenerational culture and heritage fisheries. Loss of ecosystem services to seafood working communities, where regular work and remuneration are essential to support families and businesses, can make community members more vulnerable, i.e., less resilient in coping with adverse events and adapting. A case study approach portrays how outreach materials for these communities was developed and delivered by a team of scientists in concert with community partners. Two primary tenets were considered to develop outreach and hazard communication content for communities that were both affected by the oil spill and suffered declines or collapses in their fishery resources: (1) know your audience, and (2) know your audience. Piloting of outreach materials with community partners and residents provided feedback essential to refocusing and better targeting content to foster clarity and meaningfulness for coastal residents. Community engagement in all phases of the study proved an important component of this community-based research to build trust essential to perceiving scientists as a resource, understanding how background levels of PAHs and DOSS in their seafood were not related to the spill, nor their fishery collapse, and valuing scientists' contributions to the community. Understanding different perspectives between and within communities, relative to environmental issues and perception of risk, is vital to messaging science in support of community resiliency, and contributing to the sustainable health of residents and their coastal environments. Supported, in part, by grant from the National Institute of Environmental Health Science (U19 ES020683).

60 Mining the Traditional Ecological Knowledge of Gulf of Mexico Communities to Help Unravel the Legacy of the Deepwater Horizon Oil Spill

D. Wetzel, Mote Marine Laboratory / ELF; J. Reynolds, Mote Marine Laboratory

The Deepwater Horizon oil spill tragedy happened over six years ago, with long lasting impacts on the affected environment and the people who live there. As is expected after such an environmental disaster, a number of studies have been or are being conducted to assess a range of physical, chemical and biological issues, including but not limited to factors that influenced movement and distribution of the oil, the fate of the spilled oil and dispersant, and the effects of oil and dispersant on exposed habitats and organisms. Studies have also been conducted that have addressed the socio-economic and human health impacts on the residents of the impacted areas. However, this effort, as in most environmental research, has typically occurred in the absence of any community input or even awareness. Granted, the execution of research must be done using principles of proper scientific process, which does not require an engagement of local communities. However, the coastal communities of the Gulf of Mexico are deeply dependent on healthy ecosystems and living resources for their survival and well-being, and whereas they rely less than scientists on written documents or scientific analyses, they possess unusual insights into types, extent and causes of environmental changes. They can be productive and valuable partners in developing research initiatives by providing traditional environmental knowledge (TEK) that non-local scientists will never have. Community based participatory research benefits scientists significantly by providing historic environmental information, which is usually not formally documented, but rather is orally passed down through the generations. Archival of videos and diary notes from community members collected during and after the Deepwater Horizon spill, documenting daily observations of a major spill, are yet untapped resources that can still be used to help understand the environmental impacts from the spill. A partnership between a multidisciplinary group of scientists and communities directly impacted by environmental changes takes considerable time in which to achieve trust, extra communication, and a willingness to explore alternative ways of understanding nature and the natural environment. That effort is worthwhile both scientifically and societally, to better assess and solve environmental problems, manage and regulate natural resources, and promote compelling environmental awareness.

61 Oiled Vision: Understanding Fishes' Visual Response to Oil Spills through Research and Film

E.K. Barnes, Univ of North Texas; D. DiNicola, Univ of Miami RSMAS; J.T. Magnuson, Univ of North Texas / Biology; M. Levin, Univ of North Texas / Dept of Media Arts; J. Stieglitz, Univ of Miami – RSMAS / Division of Marine Biology and Fisheries; M. Grosell, Univ of Miami / Deaprtment of Marine Biology and Ecology; A.P. Roberts, Univ of North Texas / Dept of Biological Sciences Inst of Applied Science

The Deepwater Horizon oil spill resulted in a variety of issues concerning both environmental and human health and consequently had extensive coverage by the press during clean-up efforts. Research on the ramifications of the spill is still being undertaken six years later. This project centers on a charismatic gamefish potentially affected by the spill, mahi-mahi (Coryphaena hippurus). Mahi-mahi are dependent on vision for feeding, schooling, spawning, and predator avoidance, like many other large pelagic sportfish. The immediate effects of oiling on juvenile mahi-mahi vision were the focus of research and the basis for a short film. As humans are a highly visual species, the film is designed to make the mahi dealing with visual impairment and deformities caused by oil exposure, relatable. When the eyesight of another animal is failing, the volume of the loss can be understood by the public because of how dependent humans are on vision. The film covers an aspect of the research following the Deepwater Horizon oil spill in a way that helps viewers understand the effects in a new and engaging way; through the eyes of a fish. This research was made possible by a grant from The Gulf of Mexico Research Initiative.

62 How research in oil and surfactant toxicity helped create a community outreach program in southern Louisiana

C. Green, Louisiana State Univ; J.A. Nyman, Louisiana State Univ / School of Renewable Natural Resources

Large amounts of oil spill and wetland research is virtually unintelligible to non-scientists. We are attempting to help non-scientists understand some of this research. Although no spills are preferred, the public is concerned that many current oil-spill clean-up products are only slightly better than the spill effects alone. The public also express concerns about the overall toxicity of the ecosystems they live and work in. Initially, we planned to share information with non-scientists at fishing rodeos within coastal Louisiana, however, that proved unworkable. We then prepared and posted videos on YouTube and also offered public meetings. Our outreach team conducted a series of informational workshops at libraries in Louisiana and recorded metrics of attendance and participation. These initial meetings provided incomprehensible jargon to small audiences but later meetings have been better suited and to larger audiences. Collaborations with LSU's Coastal Roots program, Louisiana SeaGrant and its network of extension agents, and creating a Facebook page to assist in advertising the YouTube videos and public meetings has increased our ability to interface with the community. The team has also conducted a two-day workshop for high school science teachers intended to help them create curriculum for oil and surfactant related topics within their courses. These workshops are a mixture of demonstrations, relevant classroom experiments, and lectures. Printed materials distributed at events complement our oral presentations and demonstrations. To date we have brochures that detail importance of fish physiology and how surfactants in everyday life and their use. We have created videos to detail how toxicologist determine acute toxicity and have created a brief video to inform people about the use of aquatic organisms in toxicology testing. We continue to be challenged when we try to teach non-scientists information, however, we believe that we have discovered what works and what does not work within our community.

63 Post-settlement conclusions of the Trustee toxicity testing program conducted in support of the Deepwater Horizon Natural Resource Damage Assessment

J.M. Morris, M.O. Krasnec, C. Lay, H. Forth, R. Takeshita, J. Lipton, Abt Associates; M.L. Gielazyn, NOAA / co USEPA Region IV

A settlement resolving civil claims in excess of $20B with BP was finalized in April 2016, six years after the Deepwater Horizon (DWH) disaster. Since 2010, we have designed, implemented and managed the Trustee toxicity testing program for the DWH Natural Resource Damage Assessment. This program included over 600 toxicological bioassays and related chemical characterizations. The objectives of the program were to determine the toxicity of DWH oil, dispersed oil, and dispersant to native and surrogate species in the Gulf of Mexico. To that end, we developed a large study matrix with over 35 species of fish and invertebrates and a variety of lethal and sublethal endpoints. Samples of DWH oil representing four distinct degrees of weathering and one dispersant (COREXIT 9500) were tested through a variety of different exposure routes including water accommodated fractions, surface sheens, direct oil exposure, sediment, and dietary. We also investigated the effects of additional environmental stressors including photo-induced toxicity. The studies were conducted by Abt Associates and NOAA scientists along with over 24 principal investigators from collaborating university, government, and private laboratories. We will discuss our more unique discoveries and how information from this program is being reported to the public through various presentations, data portals, and documents.

64 Sublethal impacts of the Deepwater Horizon oil spill on pelagic top predators from the Gulf of Mexico – communicating science to a broad audience

M. Grosell, Univ of Miami / Deaprtment of Marine Biology and Ecology; D. DiNicola, Univ of Miami RSMAS; J. Stieglitz, Univ of Miami – RSMAS / Division of Marine Biology and Fisheries; Y. Wang, RSMAS Univ of Miami; E. Mager, Univ of North Texas / Dept of Biological Sciences; D. Nelson, R.M. Heuer, Univ of North Texas; D. Crossley, Univ of North Texas / Dept of Biology; L. Schlenker, RSMAS, Univ of Miami / Marine Biology and Ecology; C. Pasparakis, Univ of Miami; P. Perrichon, Univ of North Texas; J.T. Magnuson, Univ of North Texas / Biology; L.E. Sweet, Univ of North Texas / Dept of Biological Sciences; A.P. Roberts, Univ of North Texas / Dept of Biology Inst of Applied Science; D.D. Benetti, Univ of Miami / Dept of Marine Ecosystems and Society

A wide range of sublethal effects have been observed in mahi-mahi following exposure to surface slick oil obtained from the 2010 oil spill in the Gulf of Mexico. These sublethal effects include, but are not limited to, reduced swim performance, impaired ability to avoid oil, impaired visual acuity, reduced stroke volume and cardiac output in adults as well as larvae, increased embryonic metabolic rate and energy depletion. An overview of these effects, many of which are observed after brief exposures (< 24 hours) at low and environmentally relevant PAH concentrations (< 10 µg total PAHs/l) will be presented. While these sublethal effects may impair ecological fitness and long term survival, they are difficult to relay to a broad lay audience due to the complexity of the findings. We employ a range of outreach strategies, including social media, hands-on demonstrations at various events, school visits, and web-based video media to disseminate our findings to the broader public. In addition, we are currently developing and testing a web-based interactive teaching module aimed at grades 4-12 on aerobic swim performance. In this web-based teaching module, students at various levels will be able to use real data to perform simulated experiments. As part of the simulations students will select experimental animals and experimental conditions and be introduced to data analysis and complex physiological considerations. The web-based simulation module is paired with lesson plans to assist teachers in tailoring teaching to their students' grade level and includes an assessment of the knowledge gain obtained by the students after having completed the module. The assessment is paired with detailed demographic data allowing for direct evaluation of impact of this outreach effort. This research was made possible by a grant from The Gulf of Mexico Research Initiative. Data are publicly available through the Gulf of Mexico Research Initiative Information & Data Cooperative (GRIIDC) at https://data.gulfresearchinitiative.org.

Scientific Advances Supporting Aquatic Life Water Quality Criteria Derivation – Part 1

65 An Overview of USEPA's Approach to Revising the 1985 Guidelines for Deriving Aquatic Life Criteria

M. Elias, K. Gallagher, USEPA / Office of Water

The USEPA Office of Water will provide an overview and update of the steps being taken to revise the 1985 Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses (Stephan et al. 1985). The presentation will discuss outcomes of the Invited Expert Meeting on Revising Guidelines for Deriving Numerical National Water Quality Criteria, which occurred in September of 2015 and which represented a first major step in the Guidelines revision process. Development steps that have occurred since that time will be detailed along with the planned process forward. The focus will be on a discussion of the two defining objectives that have been identified for the revision: 1) Developing a larger number of criteria more rapidly for the broader protection of aquatic life, and 2) Refining methods for deriving state-of-the-science criteria through comprehensive analyses. The first objective reflects the recognition that thousands of chemicals enter the environment from anthropogenic activities. Because rigorous testing of all chemicals is infeasible, there is a need to efficiently derive criteria using approaches to estimate safe environmental concentrations with limited empirical data. The second objective reflects that for a smaller group of chemicals, criteria development may be scientifically complex, and deriving robust criteria may require extensive study. This presentation represents an introduction and overview to the broader session, which provides an opportunity to further the discussion with the international scientific community on topics and issues relevant to the development of criteria for the protection of aquatic life.

66 Chemical standard derivation for the protection of aquatic life: A guided world tour

C.E. Schlekat, NiPERA; G. Merrington, A. Peters, wca; D. Leverett, WCA-Environment Ltd

Global regulations striving to protect aquatic ecosystems typically share goals with respect to levels of environmental protection, but fundamental differences exist within the methodologies, data selection decisions, and implementation strategies used by different jurisdictions to achieve regulatory goals. This presentation focuses on approaches used by Australia, Canada, the European Union, and the United States of America to develop chemical-specific standards intended to protect aquatic life. At the highest level, these jurisdictions agree that chemical standards play a role in maintaining biodiversity, preventing ecosystem degradation, and protecting endangered species. However, the ways in which these different jurisdictions implement chemical standards and the degree of regional heterogeneity within a given jurisdiction varies considerably. From the scientific perspective, all approaches share the use of laboratory ecotoxicity data as the basis for determining a chemical standard. The different jurisdictions vary in the consideration of laboratory ecotoxicity data at several levels, including the relative importance of acute and chronic data, appropriate statistical endpoints (e.g., preferences for EC10, EC20, No Observed Effects Concentrations, etc.), the relevance of data from specific taxonomic groups (e.g., unicellular algae), and acceptance of data from native versus non-native species. Most jurisdictions recognize probabilistic approaches like Species Sensitivity Distributions (SSDs), but minimum data requirements allowing the use of SSDs, and the models used in data analysis, show jurisdictional differences. Refined approaches accounting for the derivation of regional- and site-specific chemical standards are increasingly recognized (e.g., bioavailability-based approaches for metals), although the level of sophistication (e.g., hardness-based approaches versus full bioavailability normalization) and approaches for implementation vary. Jurisdictions also depart with approaches used to quantify and manage uncertainty, e.g., consideration of multiple lines of evidence, such as field and mesocosm data; and, the use of assessment/uncertainty factors in criteria determination. A case study comparing current approaches for determining quality standards for nickel will be used to highlight the similarities, differences, and possibilities for harmonization among these different jurisdictions.

67 Could ecological thresholds of toxicological concern (eco-TTCs) be used to support development of ambient water quality criteria?

S.E. Belanger, The Procter & Gamble Company / Environmental Stewardship and Sustainability Organization; M.G. Barron, USEPA / Gulf Ecology Division; T.J. Norberg-King, USEPA / NHEERL/Mid-Continent Ecology Division; M.R. Embry, ILSI Health & Environmental Sciences Institute

The Threshold of Toxicologic Concern (TTC) is an approach used for a decades in human hazard assessment. A TTC establishes an exposure level for a chemical below which no appreciable risk to human health is expected based upon a de minimis value for toxicity identified for many toxicologically similar chemicals. The TTC concept applied in an environmental context (eco-TTC) has been proposed to be a Predicted No-Effect Concentration (PNEC) for ecological communities and establishes a concentration expected to have a de minimis probability that effects would be observed for a given group of compounds. Chemical grouping could be defined by mode of action, functional use, or some other relevant criterion. TTCs and eco-TTCs use a form of statistical distribution analysis highly similar to that of a Species Sensitivity Distribution, except that an eco-TTC is based on distributions of PNECs for groups of compounds rather than distributions of test organism endpoints for a specific chemical. Similarly, chemical tolerance distributions (CTDs) are defined as distributions of response values (LC50's or NOECs) for a specific taxonomic units (e.g., algae, invertebrates, fish, or even specific species) within a chemical group. In this presentation we develop eco-TTCs and CTDs and compare the results to water quality criteria values for existing chemicals using approaches from the US and Canada. Based on these results we provide recommendations for what role eco-TTCs or CTDs could play in developing new ambient water quality criteria for chemicals lacking sufficient data to develop chemical-specific values. Disclaimer: This presentation does not necessarily reflect the views or the policies of the US Environmental Protection Agency.

68 Deriving Water Quality Criteria Using Mode of Action Models: ppLFER Target Lipid Model of PAH and Narcotic Toxicity as an Example

D.M. Di Toro, Univ of Delaware / Civil and Environmental Engineering

The EPA technical quidelines for developing aquatic water quality criteria were a seminal contribution. They provided a clear solution to the problem of incorporating varying species sensitivity (protecting at the 5th percentile most sensitive specie) as well as other important considerations (minimum database, frequency of compliance and duration of exposure). However they are limited to one chemical. The purpose of this paper is to suggest that this problem can be overcome by using models that apply comprehensively to all the chemicals in a single mode of action. The polyparameter target lipid model (ppTLM) is an example of such a model. There are two crucial characteristics that make this model, or, for that matter, any model, practical for criteria development. (1) The model requires a species specific parameter that is chemical independent. For the TLM, it is the critical target lipid body burden – the organism lipid normalized concentration that causes 50% mortality. It has been found to be applicable to all chemicals that have the same toxic mode of action, namely type I and II narcosis. (2) The model requires a chemical specific parameter that is species independent. For the TLM, it is the partition coefficient between the target lipid and water, KLW. It is assumed to apply to all organisms that have the same mode and site of action – namely concentrations in the target lipid that produce the same toxicity at the same molar concentration (mmol/kg lipid). It is estimated using either the octanol-water partition coefficient and chemical class corrections for the original TLM, which applies only Type I narcotics, or a polyparameter linear free energy relationship (ppLFER) that estimates KLW using a Abraham model, which applies to both Type I and Type II (polar narcotics). The ppTLM can be used to derive HC5 concentrations since the species sensitivity distribution is the probability distribution of the critical body burdens. Therefore the ppTLM can serve as a prototype for models of modes of action that share the same characteristics: species sensitivity parameter(s) that apply to all chemicals in the MoA class; and chemical binding parameter(s) that apply to all species being considered. The correct additivity model follows from these characteristics. The criteria for PAHs and other classes of narcotic chemicals will be compared to criteria derived using the single chemical EPA methodology.

69 State-of-Science Approaches to Determine Sensitive Taxa for Water Quality Criteria Derivation

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Current Ambient Water Quality Criteria (AWQC) guidelines specify pre-defined taxa diversity requirements, which has limited chemical-specific criteria development in the U.S. to less than 100 chemicals. A priori knowledge of sensitive taxa to toxicologically similar groups of chemicals should facilitate more rapid development of AWQC by focusing data collection on the most sensitive aquatic species or taxonomic groups. Two tools available for determining the sensitivity of taxa include Web-based Interspecies Correlation Estimation (Web-ICE; www3.epa.gov/webice/) and Sequence Alignment to Predict Across Species Susceptibility (SeqAPASS; https://seqapass.epa.gov/seqapass/). Web-ICE estimates acute toxicity to aquatic organisms with limited or no test data based on log-linear regression models of the sensitivity of a surrogate and predicted taxon. Web-ICE generated species sensitivity distributions can then identify sensitive taxa and estimate toxicity values. SeqAPASS determines species similarity in aligned amino acid sequences of specific molecular targets, including functional domains such as the ligand-binding domain of a biomolecule responsible for the toxic action of a chemical. Susceptible taxa are identified based on sequence similarity to a known sensitive query species. Linking these two tools would also allow for determining insensitive taxa or groups of general similarity, so that data collection efforts could be optimized towards those species that would drive criteria derivation. Application of state-of-the-science tools such as SeqAPASS and Web-ICE, along with grouping toxicologically similar chemicals by mode of action or molecular initiating event, should facilitate development of the next generation of AWQC.

70 Application of adverse outcome pathway-based tools to ambient water quality criteria development

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Increasing numbers and diversity of chemical contaminants are being detected in ambient surface waters. States, regions, and communities across the US are faced with the issue of understanding which chemicals may warrant concern and at what concentrations. Integrating new scientific data streams and predictive approaches into the process of numeric water aquatic life criteria development offers the potential to develop more criteria, for more chemicals, more rapidly. For example, well established models of non-polar narcosis provide an effective estimate of "baseline" toxicity. Emerging data streams from high throughput, pathway-based, toxicity testing programs provide broad spectrum screening for more specific modes of action that may result in deviations from "baseline" toxicity predictions (i.e., excess toxicity). The adverse outcome pathway (AOP) framework provides a scientifically credible basis for linking specific pathway-perturbations to predicted hazard and facilitates systematic consideration of taxa and life stages likely to be sensitive. Application of this information can facilitate more targeted and hypothesis-driven approaches to the collection of data for use in numeric criteria development. It can also support interim application of appropriate AOP-based prediction models in cases where empirical data are lacking. While these approaches will be subject to error and uncertainty, they offer the ability to provide a scientifically-based preliminary estimate of levels of concern, which can immediately guide priority setting and programs at the state and regional level while criteria are refined through the gradual application of stronger data sets and more tailored and sophisticated modeling. Examples illustrating how these tools can be integrated to aid rapid numeric criteria derivation will be presented. The contents of this abstract neither constitute nor necessarily reflect USEPA policy.

71 Potential Applications of Weight of Evidence Methods to Water Quality Criteria

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When more than one piece of evidence is available to address a problem, it may be appropriate to weigh the evidence. Evidence may be weighed to derive quantities or qualities. When multiple estimates are available for the same quantity, the quantitative weighing is termed meta-analysis. Currently, when deriving water quality criteria, multiple test results for a species and multiple tests of species in a genus are combined as geometric means. One might quantitatively weight those values before averaging, as in inverse variance weighting. Qualitative weight of evidence may be used, as with human health benchmarks, to determine what mode of action or route of exposure is applicable (e.g., is it an endocrine disrupter; is dietary exposure significant). When deriving criteria using field data, qualitative weight of evidence may be used to determine whether the exposure-response relationship is causal and whether it is significantly confounded. It may also be used to determine whether a field-derived value for one region is applicable to another. The ultimate application of weight of evidence would be to choose among or combine potential criteria derived by different methods (e.g., conventional toxicity tests, microcosms, mesocosms, field surveys, or models). Finally, weight is the opposite of uncertainty. Therefore, qualitative weighting of a body of evidence, based on consideration of relevance and reliability, can be used as an expression of the confidence in a value or conclusion that goes beyond the scatter that is quantified by statistical uncertainty. The views expressed in this abstract are those of the authors and do not necessarily represent the views or policies of the USEPA.

72 A Retrospective Evaluation of the USEPA's Guidelines for Ambient Water Quality Criteria Development Given What We Now Know

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Many of the USEPA's ambient water quality criteria (AWQC) were initially developed in the 1970s and early 1980s, and the USEPA's current guidance for deriving AWQC was released in 1985. Over the last 30+ years, AWQC for a large number of constituents have been either newly developed or updated. The large increase in toxicity datasets now available for a large number of constituents provides an empirical basis for assessing the 1985 AWQC derivation guidance and whether any aspects of guidance should be considered for modification. We conducted a retrospective evaluation of criteria and toxicity data for representative constituents in different chemical classes to evaluate several questions, such as: (1) Are the minimum data requirements for taxonomic diversity (i.e., the "8-family rule") adequate given what we now know from the much broader empirical toxicity data available? (2) Should the minimum data requirements vary by chemical class given our understanding of mode of action? (3) Should end points beyond survival, growth, development, and reproduction be more explicitly considered in criteria development rather than inconsistently evaluated as "other data"? In this presentation we will provide our current thinking on these and other topics pertinent to the question of whether updates to our 31-year old Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses should be considered based on what we have learned in the intervening years.

Advancing -Omics into Regulatory Frameworks: Case Studies and Perspectives

73 Applying population genomics to environmental regulations

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Differences in inter-individual response are described by variation in environments and genomes that combine to give rise to phenotypic variation observed in populations. Environmental genomics provides tools for understanding these features that offer links to phenotypes, which are in-line with proposed changes in regulatory toxicology. In this talk we draw from recent environmental genomic case studies to explore how environmental stress contributes to genome variability, influences the fate of genetic variation in populations, and over micro-evolutionary time scales determines the fate of phenotypes. These studies contribute to and make use of maturing genomic tool kits for the killifish, Fundulus heteroclitus, and the water flea, Daphnia pulicaria. Using these animal models we explore how functional variation in gene expression and gene regulatory networks contributes to phenotypic plasticity, and influence homeostasis in response to environmental change, and how environment-induced alterations in the magnitude and distribution of gene copy number (CNV) in natural populations contributes to adaptations to extreme environments. We will discuss the importance of understanding genome variation and the evolutionary forces that shape it in light of environmental regulations.

74 How consistent are we? Inter-laboratory comparison for male fathead minnows exposed to 17α-ethinylestradiol

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Transcriptomic approaches are widely used to examine effects of aquatic contaminants in both laboratory and field studies. Fundamental questions remain however for defining the limits of the technology and how it may be used in environmental monitoring programs. Uncertainties exist as to how molecular initiating events translate into adverse effects at the population level, as well as how large a magnitude or transcriptome response constitutes an adverse effect (threshold). Also debated are the most appropriate metrics for quantifying an “omics” response (e.g. fold change, intensity, p-value, pathway, or network). If omics technologies are to be established in governmental programs for environmental monitoring and risk assessment, they must adhere to rigorous standardization that ensures reliability and consistency. To investigate this, male fathead minnows (FHM), a widely used aquatic species for toxicity testing, were exposed to 25 ng/L EE2 for 96 h, and six independent laboratories received frozen livers to conduct a transcriptome analysis. Independent laboratories were free to use different processing and analysis packages to analyze the dataset. Data were also analyzed using a common method. Congruence was ~50% across laboratories when it came to identifying differentially expressed targets. This was not surprising given the flexibility in methods used to detect differentially expressed transcripts. The range in the magnitude of response of the transcriptome was variable across laboratories, with some laboratories reporting a smaller dynamic range than others for transcript fold change. However, when transcripts were ranked by fold change across laboratories, there was a strong positive relationship ($R2 = >0.9$, $p< 0.001$). Most important from a regulatory or monitoring standpoint was that, when a core group of estrogen responsive genes were singled out and located in each dataset, all laboratories save few exceptions were consistent in identifying the genes as differentially expressed; the laboratories also showed high congruence in estimating magnitude of response across a range of fold changes. This was true regardless of whether a single unified approach or independent data analyses were used. These transcripts were selected to encompass a range of fold change responses from dramatic (>20-fold) (e.g. vitellogenin, estrogen receptor 1) to more subtle a response (< 1.5 fold) (i.e. BOP1, Block of Proliferation 1). These data suggest that transcripts responsive to a chemical stressor can be consistently identified across laboratories, in designs that allow flexibility in the methods used. The results of this study suggest that omics technologies can be used to advance environmental monitoring efforts under a yet to be developed standardization framework.

75 Industrial perspective on environmental genomics for monitoring biodiversity and key species

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The oil and gas industry uses biodiversity assessments to determine baseline conditions and potential impacts during operations. These assessments are often part of a larger environmental monitoring program and vary according to application, but in general, the environmental monitoring programs are labor- and time-intensive. For example, with offshore drilling operations, benthic sediments are partly characterized by isolating organisms via sieving large grab samples and identifying them. This process is costly, time-consuming, and reliant on the availability of qualified taxonomists. Alternative methods for biodiversity assessments would alleviate some of the cost and time pressures and provide a safety net in the event the traditional analyses are inconclusive. One such alternative method is the analysis of environmental DNA (eDNA), which refers to DNA extracted from environmental samples from organisms that inhabit or shed cells and waste in that. We are currently assessing the potential for eDNA methods to provide equivalent or supplementary information as traditional environmental monitoring methods. The analysis of eDNA permits the detection and identification of eukaryotic and/or prokaryotic organisms without direct observation or capture. Molecular approaches like DNA barcoding and eDNA could potentially be less expensive, safer, and more comprehensive than traditional monitoring methods. Shell is interested in the eDNA approach for biodiversity assessments and for species monitoring, and it has undertaken preliminary research to determine the utility and applicability of eDNA analysis. This presentation will discuss the pilot projects using eDNA for sediment assessment and species monitoring. Based on these analyses, eDNA could be used for supplementing traditional monitoring programs. There are several limitations to this approach, such as the biases introduced by PCR amplification, the reliance on the barcoding database for species identification, and the uncertain provenance and longevity of DNA. The promise and limitations of this approach will be discussed from an industry perspective.

76 Transcriptional (RNA-seq) and metabolic (MS/MS) profiling reveals host-microbiome interactions during adaptation to chronic pesticide exposure

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Chronic pesticide exposure affects over one hundred million people world wide each year, and costs in excess of $1.2B per year in the United States alone. In metazoans, chemical exposures impact both the host and the microbiome. The host genome encodes several thousand enzymes, while the microbiome, in higher bilaterians, encodes millions, and many chemicals, including collateral dietary pesticides, resist host metabolism and are transformed exclusively by microbes. Hence, health affects of pesticide exposures may be moderated by microbial metabolism. We applied a multi-omics, systems biology approach to map host-microbiome interactions during chronic pesticide exposure in two distant metazoans, the model organisms Mus musculus and Drosophila melanogaster. We studied the widely used herbicides paraquat, atrazine and glyphosate, and observed changes in host gene expression, microbiome community architecture, and the gut metabolome that occur during low (10X DWEL) and high (1/10th LC50) dietary exposures. In both species, all three compounds remodel gut microbiomes (16S and metagenomic profiling). In mouse, impacts on the gut microbiome are dependent on host genetic background: GWAS on microbial abundances reveals that numerous microbiome quantitative trait loci (mbQTLs) are coincident with genes involved in inflammatory bowel diseases and cancer. Some microbial clades are completely extinguished by herbicide exposure, and these are replaced by expansions of microbes with overlapping metabolic strategies. In both species, atrazine disrupts expression programs (RNA-seq) for genes involved in cholesterol and lipid biosynthesis and transport, and metabolomics (MS/MS) analysis reveals that these disruptions affect numerous glycerophosphocholines. Therefore, the impacts of atrazine exposure on some biosynthetic processes are conserved between mouse and fly, across 600 Myr of evolution and in radically different microbial backgrounds. Further, all pesticide exposures resulted in neuromuscular phenotypes detectable through machine learning on open-arena behavioral imaging data. This analysis, though preliminary, already points toward both metabolic and transcriptional markers that could be screened in human and other chordate populations exposed to pesticides.

77 Developmental neurotoxic effects of pesticides, MeHg, and PFHxS combining behavior and cognitive studies with metabolomics and proteomics

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Worldwide, serious concern has arisen about the increased incidence of learning and developmental disorders in children. Various recent epidemiological studies have indicated that exposure to low doses of environmental biologically active contaminants during human development can have deleterious effects on cognitive development in childhood. The EU-funded project DENAMIC investigates neurotoxic effects (e.g. learning and developmental disorders) of low-concentration mixtures of pesticides and a number of common environmental pollutants in children. In the current study the aim was to investigate the behaviour and cognitive effects of pesticides, methylmercury, and perfluorohexane sulfonate (PFHxS) exposure in mice and to further study the underlying molecular mechanisms of the observed effects using metabolomics and proteomics. Male mice were exposed to a single dose of pesticides (chlorpyrifos, carbaryl, cypermethrin, endosuldfan), methylmercury or PFHxS at postnatal day 10. Behaviour effects were studied at the adult age of 2 to 4 months. The studies showed that all chemicals can cause developmental neurotoxic effects, even after a single exposure which was given at a vulnerable period of brain development. The cognitive and behaviour tests showed that the early life exposure of the compounds can alter adult spontaneous behavior and cognitive function. Interesting the chemicals had different modes of action causing the same apical endpoint (increased spontaneous behaviour). Targeted and untargeted metabolomics and proteomics studies were carried out in brain tissues of cerebral cortex and hippocampus. Biochemical networks were mapped for each brain tissue using MetaMapp and Cytoscape. Metabolomic and proteomic analysis showed that a limited number of metabolites and proteins were down or upregulated, and that most were regulated in hippocampus. The strongest effect was found for PFHxS. Regulated metabolites and proteins were mostly related to axons/neurons, mitochondria, purine pathway, NADPH/NAPD activity, ATP/ribonucleotide metabolic processes. In general, the results showed that the cholinergic system was affected. A positive correlation was found between the levels of the neurotransmitter acetylcholine and acetylcarnitine in hippocampus; it has been suggested in literature that an increase of acetylcarnitine can give a spontaneous release of acetylcholine. The developmental toxic effects might cause neurological/neurodegenerative disorders/diseases.

78 Metabolomics in the Assessment of the Health of Sentinel Species in the Great Lakes

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Pollutants enter the Great Lakes in Southern Ontario through a variety of sources including wastewater treatment plants (WWTP) and local industries. A diverse set of contaminants has been detected within this environment including metals, pharmaceuticals, PCBs, PAHs and pesticides. The number and range of such compounds presents a significant challenge in both monitoring the effectiveness of regulatory changes implemented to reduce pollution loads and in assessing biological effects and risks. Transcriptomic, proteomic, and metabolomic measurements of sentinel species that have been exposed to environmental samples from affected sites provide an opportunity to evaluate early biological impacts in a controlled laboratory setting and to correlate these biological effects with measured contaminant levels. Such data can provide valuable insight into prioritizing and refining future contaminant measurement strategies, and can inform regulatory approaches. In this set of studies, rainbow trout and hexagenia sp. were exposed to surface water, effluent, and sediment (hexagenia only) collected at various sites and WWTPs in Lake Erie and Lake Ontario. Exposures were performed in a laboratory setting with each exposure lasting 48 hours. Concentration values of 219 metabolites, including amino acids, biogenic amines, ∑hexose, fatty acids, bile acids, acylcarnitines, sphingomyelins, and glycerophospholipids, were measured in rainbow trout liver and whole hexagenia. In parallel, concentrations of PCBs, PAHs, metals, and a diverse set of environmental contaminants were measured in the exposure media. Univariate and multivariate statistical techniques, such as ANOVA, PCA, and OPLS-DA, were used to identify metabolites varying between the different location/concentration groups and to correlate metabolite changes with available water quality and contaminant concentration data. Preliminary analysis indicates that metabolomic differences can be identified in rainbow trout and hexagenia exposed to control samples compared with those exposed to effluent. Furthermore, the metabolomic effects of effluent from specific sites within Lake Ontario are distinct. Likewise, hexagenia sediment exposures show distinct metabolomic differences after exposure to field samples versus exposure to clean sediment. This work is part of a multiomic study on exposure and effects of industrial and urban inputs in the Great Lakes.

79 Transcriptomic response of Mytilus larvae to simultaneous copper and ocean acidification exposure

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Future changes in ocean chemistry will have an unclear effect on metal toxicity to marine organisms. In particular, very little is known about the combined effects of ocean acidification and metal exposure at the molecular level. High throughput sequencing technologies will allow us to screen for robust biomarkers of metal toxicity that are effective even as ocean acidification progresses. Our past work has identified transcriptional biomarkers of copper exposure in mussel larvae and adults. This research revealed a set of genes with dose-responsive expression that are indicative of copper exposure and correlate well with morphological abnormality. In the current study we tested the consistency of known copper-responsive transcriptional profiles under simulated ocean acidification, and explored novel biomarkers of co-exposure to copper and ocean acidification. We exposed California mussel (Mytilus californianus) embryos to a range of seven copper concentrations and two CO_2 concentrations (400 ppm and 800 ppm) for 48 hours, the crucial time period of early development. At 48 hours, larval survival and proportion of normal development were determined. 30 larval samples spanning our range of copper and CO_2 concentrations were sequenced on an Illumina HiSeq 4000. Both copper and CO_2 concentrations influenced survival and normal development, and animals slightly benefited from exposure to elevated CO_2 concentrations. The transcriptomes revealed that dose-responsive gene expression profiles were evident at both high and low CO_2 concentrations. While many genes exhibited consistent expression profiles in both CO_2 treatments, a subset of genes responded in a unique way to copper stress under future OA conditions. High throughput expression data like this will be vital to develop appropriate and reliable toxicity assays that will still be effective in coming decades.

80 Characterization of gene regulatory networks that coordinate early adaptive response to environmental stress in Daphnia magna

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The modulation of mRNA levels through transcriptional and post-transcriptional regulation is among the earliest cellular responses to changing environmental conditions. We analyzed the immediate-early response of three Daphnia magna genotypes to 12 environmental challenges, including 6 biotic and 6 abiotic stressors, lasting between 4 to 24 hours via a genome-wide transcription profiling (RNA-seq). Each exposure was imposed to mimic ecologically relevant conditions that Daphnia encounters in the natural environment, and hence are within the known adaptive, non-toxic regimes for this organism. We find that most differentially expressed genes are modulated in a genotype-specific manner and in response to only one or a few conditions. However, genes are organized into co-responsive modules, and these are coherently co-expressed across numerous conditions and genetic backgrounds. Transcripts associated with adaptation to genotoxic conditions provide straightforward biomarkers of abiotic perturbations, and the specific cohort of responsive genes provides insight into the mode of action of the toxicant. Remarkably, we find transcriptional signatures of diapause in response to stressors as early as 4 hours, indicating that the decision to generate ephippial eggs is made almost immediately upon the transduction of environmental challenges. Condition-specific transcriptional modules also provide an opportunity to discover genes of previously unknown functions, as they are broadly enriched for poorly conserved or recently evolved genes, and this study constitutes the first evidence of biological rolls for several hundred genes. This survey of stress response in a keystone ecological species provides insight into the gene regulatory networks that coordinate early (< 24h) adaptive response to environmental stress.

Status and Trends of the Landscape-Scale Mercury Problem in South Florida and the Everglades

81 History, Trends and Management Implications of Florida's Mercury Problem

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Mercury (Hg) bioaccumulation in fish and wildlife from the Everglades has been a concern since the mid 1970's when first identified as an issue within Everglades National Park. Concerns expanded during the 1980's as sport fish with Hg concentrations well over 1 mg/kg were found within the Everglades Protection Area (EPA). Elevated Hg concentrations in fish and wildlife are an important endpoint within the Everglades mercury cycle and reflect relatively high rates of atmospheric deposition of inorganic Hg coupled with wetland and coastal ecosystems that efficiently produce methylmercury (MeHg). MeHg, the more toxic form of Hg, Bioaccumulates efficiently in aquatic food webs reaching levels of concern to human and wildlife health. In 1992, an Interagency Mercury Science Program, consisting of federal, state, and private entities, was tasked with informing managers on Everglades restoration strategies to prevent a worsening of the Hg problem. An important component of this program, monitoring of fish and other vertebrate species, revealed significant declines in Hg biomagnification during the late 1990's – early 2000s. Yet concentrations remain at levels exceeding criteria for the protection of human health and within the range of potential effects to fish eating wildlife both within the EPA and downstream estuaries. Moreover, spatial gradients in water quality and biogeochemistry continue to produce "hotspots" where both the production and bioavailability of MeHg favor efficient bioaccumulation. Surveys of Hg in fish and wildlife of south Florida within the past five years have revealed Hg concentrations as high as 3.31 mg/kg in the muscle tissue of a largemouth bass (Micropterus salmoides), 4.5 mg/kg in a blacktip shark (Carcharhinus limbatus), 93.6 mg/kg in feathers of osprey (Pandion haliaetus carolinensis), and 100 mg/kg in fur of a panther (Puma concolor coryi). In 2013, the Florida Department of Environmental Protection developed a state-wide, Total Maximum Daily Load (TMDL) for Hg that called for an 86% reduction of anthropogenic emissions for the protection of human and wildlife health. Currently the primary mitigation strategy in the Everglades is through consumption advisories for fish and wildlife which urge reduced consumption of 13 freshwater species, >69 marine species, and 2 wildlife species. Future management approaches should be sought that incorporate the spatial, temporal, and ecological variability of Hg biogeochemistry.

82 The Contribution of Emissions Sources and Atmospheric Deposition to Mercury in South Florida and the Everglades

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Atmospheric deposition constitutes a potentially major source of loading of mercury to terrestrial and aquatic ecosystems in South Florida. The three main forms of inorganic atmospheric mercury are emitted in different fractions from various anthropogenic and biogenic sources and have different wet and dry deposition characteristics. The deposition contribution to mercury contamination in the Everglades is due to a combination of mercury emitted from local, regional and other world-wide emission sources. In particular, gaseous elemental mercury undergoes long-range transport and is converted to gaseous oxidized mercury through various mechanisms such as in thunderstorms in Florida. The resulting divalent mercury undergoes rapid deposition over South Florida and gets methylated to organic mercury, thus contributing to the mercury contamination in the Everglades. The paper will summarize our current understanding of the relative contributions of emission sources to mercury deposition in South Florida through an examination of both modeling results and monitoring data for wet and dry deposition.

83 Everglades Human Health Risk Assessment for Mercury

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Human exposure to mercury, a neurotoxicant, is primarily from consuming fish contaminated with methylmercury (MeHg). MeHg which is far more bioaccumulative than is inorganic mercury, is most frequently produced in water bodies from inorganic mercury being converted by naturally-occurring sulfate-reducing bacteria to MeHg; MeHg then concentrates up the aquatic food chain. Mercury is by far the leading cause for fish consumption advisories in the USA and in Florida. Current fish consumption advisories for the Everglades recommend that anglers limit and, in some cases refrain, from consuming 13 freshwater species, several marine and estuarine fish species, pig frogs and alligators. Recreational anglers who eat their catch are a subpopulation potentially at increased risk of mercury exposure. Current fish consumption advisories may not be sufficiently protective for those that consume higher than average amounts of fish and game, including some ethnic groups and subsistence fishers. This issue is particularly relevant in Florida with its widespread, elevated fish mercury levels. Accordingly, there is a need to better quantify their exposure to accurately assess the human health risks. Previous studies done in Florida took place in the 1990s and focused on fisher people in the freshwater Everglades. Yet, in southwest Florida, consumption of marine species by recreational anglers is expected to significantly outweigh consumption of freshwater species over the long term. In this presentation, human health risk assessments for various scenarios for consumption of Everglades fish and wildlife will be discussed. As well, possible explanations for the elevated mercury levels in Florida fish as these relate to options for managing human exposure to MeHg will be presented. Additionally, current State efforts to limit anglers' exposure to MeHg along with possible additional options will be considered.

84 Everglades REMAP 2014: Findings for Mercury and Other Biogeochemically Related Pollutants

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The Everglades Regional Environmental Monitoring and Assessment Program (REMAP) is a probability-based, multi-media survey that the U.S. Environmental Protection Agency (EPA) has conducted to describe, explain, and predict conditions throughout the public Everglades freshwater flow-way since 1995. The latest survey was in the wet season of 2014, when we sampled 119 locations. In addition to mercury, sulfur and phosphorus have been constituents of concern in REMAP because of their relationship to mercury toxicity and bioaccumulation, among other effects. In 2014 there was half as much methyl mercury in surface water as in the previous complete survey, done in 2005. (2014 median = 0.099 ng/l, 2005 = 0.200). There was also less total mercury in surface water, but not in proportion to the difference in methyl mercury (total mercury median of 1.5 ng/l vs. 2.1). The decline in total mercury probably reflected regional and global declines in atmospheric deposition. The mosquitofish (Gambusia affinis), a forage fish ubiquitous in the Everglades, is used in REMAP to assess mercury levels in consumers. In 2014 there was a little over one-third as much mercury in mosquitofish system-wide compared to 2005 (median of 33 ng/g vs. 87). Mercury bioaccumulation hotspots remained, with 13% of the system above EPA's predator protection level of 77 ng/g. In 2014, 26% of the greater Everglades study area had sulfate concentrations in surface water in the range reported to stimulate mercury methylation (2 – 20 mg/l), contrasting with 37% in 2005. Only 63% of the area had < 1 mg/l, which is the Comprehensive Everglades Restoration Plan goal, and only 18% remained at background level (0.022 mg/l, the REMAP analytical method detection limit). Total phosphorus in soil was unchanged, with the median value being 390 mg/kg. REMAP data show that parts of the Everglades with high phosphorus and sulfur may favor mercury methylation, but that they may also have food webs that are sufficiently degraded by these pollutants so as to inhibit mercury bioaccumulation. The remaining mercury hotspots represent places in the landscape where both processes are enhanced.

85 Contrasting Biogeochemical Conditions at Two Everglades Locations with Historically Elevated Mosquitofish (Gambusia holbrooki) Mercury Concentrations

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Southern Water Conservation Area (WCA)-2A and northern WCA-3A contain similar ridge and slough marsh communities, characterized by low P concentrations and abundant calcareous periphyton. However, marked biogeochemical differences exist between the two regions, which may affect the active mercury (Hg)/methylmercury (MeHg) cycling processes in each area. Both regions at times have been considered "hot spots", since elevated mercury concentrations have been measured in fish, though with considerable temporal variability. We compared biogeochemical factors (i.e., Hg, MeHg, sulfate (SO_4), iron (Fe), and dissolved organic carbon (DOC) concentrations) between and within monitoring locations in these regions: U3 (WCA-2A) and DB-15 (in NW WCA-3A), where a long record exists of elevated Gambusia Hg. Surface waters (SW) at U3 had higher dissolved (diss) Hg (1.0–2.3 ng/L) and diss MeHg (0.1–1.0 ng/L) than at DB-15 (0.5–2.4 ng/L diss Hg; < 0.6 ng/L diss MeHg). In marked contrast, detritus (floc) MeHg averaged 2.2-6.1 ng/g dry at DB-15 and 0.5-2 ng/g dry at U3, and was related to Gambusia tissue Hg. Typically, SW DOC was higher at U3 (23-57 mg/L) than DB-15 (14-37 mg/L), and was among the strongest explanatory variables for MeHg in water and biotic matrices (e.g., floc, Gambusia) within and between sites. However, the aromaticity of DOC was not correlated with SW Hg or MeHg concentrations at either site. Sulfate was much higher at U3 (15-32 mg/L) than DB-15 (< 1.5 mg/L SO_4). Concomitantly, at U3 porewater (PW) sulfide was elevated (1.6-4.9 mg/L) and oxidation reduction potential (ORP) was depressed (about -100 mV) compared to DB-15 (< 0.2 mg/L and about 0 mV, respectively). However, neither detrital MeHg nor SW dissolved MeHg were correlated to SW sulfate concentration at either site. Diss Fe, especially in PW, was much higher at DB-15 (1-4 mg/L) than U3 (< 0.04 mg/L), but was not correlated with MeHg at either site. The elevated Hg concentrations documented in biotic matrices at both U3 and DB-15 demonstrate that Hg methylation and bioaccumulation can occur under diverse biogeochemical conditions, particularly with respect to sulfate availability. This detailed temporal assessment shows a strong linkage between DOC concentrations and SW Hg and MeHg concentrations within and among sites. In addition, our preliminary data suggest that food web and other ecological differences exist between the two sites, which may contribute to higher biotic Hg concentrations at DB-15.

86 Sulfate in the Everglades – Sources and the effect of source reductions on Gambusia Hg concentrations

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Elevated concentrations of mercury (Hg) in a broad spectrum of Everglades biota have long been recognized as environmental and public health concerns. While the Hg problem ultimately is driven by atmospheric inputs, its biogeochemical cycling and trophic transfer is governed by a variety of physical, chemical and biological factors. Similar to many aquatic ecosystems, key amongst those factors are dissolved organic carbon (DOC) and sulfate. Sulfate dynamics can play a critical role in Hg cycling because methylation of Hg in freshwater aquatic ecosystems is governed in large part by the activity of sulfate-reducing bacteria that use sulfate as the terminal electron acceptor to metabolize organic matter under anaerobic conditions (Gilmour, 2011). Atmospheric emissions source-receptor modeling indicates that only a small fraction of Hg deposited in the Everglades originates within Florida, and the occurrence of DOC is a ubiquitous and natural feature of the Everglades landscape. Sulfate concentrations however are greatly perturbed relative to pre-anthropogenic levels, and reflect enhanced contributions from atmospheric emissions of SO_2 and land management practices using sulfate as a soil amendment in the EAA draining into the Everglades. Thus – of the three key variables governing Hg cycling in the Everglades – controlling sulfate is perhaps the only viable option for mitigating the Hg problem. This paper has two major components. First, I use solute tracers to identify and quantify the contributions of a third source of sulfate to the Everglades – viz. discharges of connate seawater into major Everglades canals draining the EAA. This source, which relates to the hydrologic alterations and practices actively used within the EAA, is thus an anthropogenic source which must be considered in evaluating sulfate control strategies. For the second component of this paper, I explore the sulfate management question by using a structural equation model to predict the effect of various reductions of sulfate on expected Hg concentrations in Gambusia spp. across the Everglades. This model, which was developed by Pollman (2014), incorporates two critical features that necessarily must be included in any statistical model of Hg cycling – the non-linearity between sulfate and methylation; and mediated inter-variable relationships. Predicted changes in Gambusia Hg will be examined both spatially, and with respect to overall shifts in cumulative distribution.

87 Trends, Hotspots and driving factors of mercury within the Everglades Ecosystem

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Mercury (Hg) methylation and bioaccumulation is a major environmental issue in the Greater Everglades Ecosystem since the late 1970s. Areas of elevation Hg concentrations in fish, soil and water can be found through the Everglades system. These areas have been operationally defined as hotspots however no formal, data driven criteria has been developed to define Hg accumulation hotspots. Biological hotspots can be defined as Hg tissue concentrations above a particular wildlife tissue threshold/criteria. Alternatively, hotspots can statistical be defined using geostatistical methods to delineate hotspot occurrence and extent, however this approach is data intensive. This presentation will discuss general trends and patterns of Hg concentrations observed in biotia, atmospheric wet deposition and surface water within the freshwater Everglades Ecosystem. Also this presentation will discuss biological hotspot identification and biogeochemical cycling of Hg. Since Water Year (May 1 – April 30) 1989 to present, Hg tissue concentrations in Largemouth Bass (Micropterus salmoides L.) has been highly variable with no significant trend apparent from sampling locations across the EPA. Meanwhile, lower trophic level species including sunfish (Lepomis spp.) and mosquitofish (Gambusia spp.) Hg tissue concentrations remain relatively high and variable with several stations experiencing significant increases in sunfish tissue Hg concentration and decreasing trends seen in mosquitofish Hg concentration. Meanwhile, wet deposition of Hg, the primary source of Hg, has remained relatively constant through the period of record. Recent studies have discussed geostatistical identification of soil Hg hotspots within the Everglades ecosystem, nutrient stoichiometric control of biological hotspots and the interaction of sulfur, iron and organic carbon within hotspot and non-hotspot locations. Although the state of Florida has adopted a statewide Hg TMDL, more work is needed to better understand and predict Hg dynamics, especially in the context of water quality conditions within the Everglades ecosystem. However, without a quantum step in our ability to link surface water conditions, microbial dynamics, ambient methyl-Hg and biota Hg levels, there is no way to justify any complimentary ecosystem-wide, parameter specific management strategy to reduce Hg risks.

88 Reduction of Sulfate Loading May Mitigate Methylmercury Production in the Everglades

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Unnaturally high sulfate loading to the Everglades is one of the key factors controlling methymercury (MeHg) production and bioaccumulation in the ecosystem. Background concentrations of sulfate in surface water of the Everglades are typically very low (0.05 – 1 mg/L) and do not support significant levels of microbial sulfate reduction and mercury methylation. Discharge of canal water containing high levels of sulfate (60-70 mg/L long term average) into the Everglades, however, has elevated sulfate concentrations in at least 60% of the ecosystem. The elevated sulfate levels stimulate sulfate reduction and MeHg production. The sulfate contamination originates from the Everglades Agricultural Area (EAA) from agricultural use of sulfur, organic soil oxidation, and other sources. Modeling, and field and experimental studies suggest that reducing sulfate loading can have dramatic impacts on MeHg production in the ecosystem. A modified version of the Everglades Landscape Model that incorporates sulfate and MeHg production modules has shown that a 37% reduction in sulfate loading to the Everglades dramatically reduces the risk of MeHg production by about 20-50% in large areas of the Water Conservation Areas and Everglades National Park (ENP). Greater reductions of sulfate have even more significant impacts on MeHg risk. Between 1999-2002, an area of the central Everglades, formerly a "hot spot" for MeHg production accumulation in fish and wading birds, saw a reduction in sulfate levels from 10 mg/L to background levels (0.05 mg/L) due to rerouting of water accompanying Everglades restoration. In response to the decrease in sulfate concentrations, MeHg levels in water decreased dramatically from 0.8 to 0.1 ng/L, with accompanying changes in MeHg levels in fish and birds. Experimental studies using lab microcosms and field mesocosms also demonstrate that reducing sulfate loading reduces MeHg production. Approaches to reduce sulfate loading to the ecosystem must be multifaceted, and include: (1) Best management Practices (BMPs) on sulfate use in agriculture in the EAA, (2) approaches for reducing organic soil oxidation in the EAA, (3) reducing intrusion of sulfate-rich groundwater, especially in cracked canal bottoms, (4) modifying stormwater treatment areas for more efficient removal of sulfate, and (5) transporting sulfate-contaminated canal water by sheet flow through marsh areas to sensitive parts of the ecosystem (e.g. ENP).

Soil Contaminants: Fate, Bioavailability, Environmental Toxicology and Risk Assessment – Part 1

89 Assessing the ecotoxicity of constituents in dredged marine sediment contaminated with TPH and heavy metals for its beneficial reuse as soil

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A significant amount of dredged marine sediment is produced worldwide by navigation dredging. The dredged sediment can be reused in many ways such as base materials for construction fill and habitat creation. Prior to the beneficial reuse of the dredged marine sediment in terrestrial environment, any potential negative effects of natural and anthropogenic constituents in the sediment on terrestrial receptors should be evaluated and if needed, the sediment should be treated to minimize the effects. This study considered salinity, TPH and heavy metals as constituents in dredged marine sediment potentially toxic to terrestrial receptors. Each constituent was sequentially removed from a dredged marine sediment sample highly impacted by TPH and heavy metals to determine the significance of each as eco-toxicological parameters. The salinity was removed by washing the sediment with deionized water (2 hr, 180 rpm, 1:5 solid-liquid ratio) and TPH was removed by solvent extraction (20 min, 180rpm, 1:6 soild-liquid ratio) and thermal desportion (300?, 1 hr). Heavy metals were removed by a soil washing procedure (1.5 M H_2SO_4, 1:3 soil liquid ratio). After the treatment, the sediment was analyzed for concentration of salinity, TPH and heavy metals, and heavy metal speciation. A toxicity test using Hordeum vulgare was conducted to assess the effect of each constituent on terrestrial receptors after sequential remediation of the sediment. Based on the toxicity test, the salinity reduction in sediment resulted in significant increase in germination and growth of H.vulgare, but the removal of TPH by solvent extraction did not have a positive effect. The toxicity test using H. vulgare after TPH removal by thermal treatment and heavy metal removal by acid treatment is underway to assess the effect of each treatment. Acknowledgement: This study received substantial support from the Geo-Advanced Innovative Action (GAIA) Project of the Korea Environmental Industry & Technology Institute (KEITI).

90 Screening-level ecological risk assessment for beneficial reuse of dredged marine sediment

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Researches on beneficial reuse of dredged marine sediment has been developed recently. It is important to estimate the environmental impact of dredged marine sediment on terrestrial ecosystem, however the ecological risk of dredged marine sediment has been rarely assessed. In this study, screening-level ecological risk assessment was conducted in order to beneficially reuse dredged marine sediment as soil. Heavy metals (i.e., Cu, Zn, Cd, Pb, Cr, Ni) which are commonly found in dredged marine sediment were selected as target comtaminants. Toxicological data of heavy metals were collected from ECOTOX of US Environmental Protection Agency (USEPA), Canadian Council of Ministers of the Environment (CCME) and some technical papers. Screening process was conducted in order to verify the quality of toxicological information regarding the statement of experimental conditions, endpoint reported, and the statistical analysis used. Stochastic approach (for Cu, Zn, and Cd) and deterministic approach (for Pb, Cr, and Ni) were both used according to the number of screened toxicological information in order to calculate predicted no effect concentration (PNEC). The PNEC values are suggested as the range with the uncertainty factor from 1 to 5. Calculated PNEC values of Cu, Zn, Cd, Pb, Cr, Ni are 17.8 – 39.7, 45.9 – 102.7, 2.3 – 5.2, 16.7 – 37.5, 12.3 – 27.5, 16.7 – 37.5 mg/kg, respectively. This study derived hazardous quotients of the six heavy metals using PNEC value and site-specific predicted environmental concentration (PEC) of each heavy metal. The hazard quotient (HQ) of Cu, Zn, Cr, Pb and Ni were higher than unity indicating a possibility of ecological risk of the five heavy metals when the dredged marine sediment is applied in terrestrial environment. Accordingly, remediation processes and a higher-level ecological risk assessment would be needed for the recycling of the dredged marine sediment.

91 Human exposure to wastewater-derived pharmaceuticals in fresh produce: Data from a randomized controlled clinical trial

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Fresh water scarcity has led to increased use of treated wastewater as an alternative source for crop irrigation. Concerns have been raised regarding pharmaceutical exposure via treated wastewater. Our aim was to assess whether carbamazepine, an anticonvulsant drug highly persistent in wastewater, is present in commercially available treated wastewater-irrigated produce, and whether human exposure to carbamazepine occurs via ingestion of this produce. In this study we follow the exposure path: wastewater --> irrigation water --> soil --> crops --> consumers. We recruited 34 volunteers aged 18-63 years (20 women and 14 men) to a single blind crossover trial. Group I (n=22) was randomized to receive treated wastewater-produce followed by fresh water-irrigated produce; Group II (n=12) received fresh water irrigated-produce followed by supermarket-purchased produce. Each exposure period lasted one week. During the trial, subjects filled food frequency questionnaires and provided urine samples. Carbamazepine and metabolite levels were measured in produce and human urine, following development of a novel method to extract and measure metabolites in urine matrix. We assessed the proportion of individuals with urinary levels above the LOD or LOQ and area under the curve over the study period. Treated wastewater-irrigated produce exhibited substantially higher carbamazepine levels than fresh water-irrigated produce. At baseline, urinary carbamazepine was undetectable in 13, between LOD and LOQ in 12, and >LOQ in 9 subjects. Following seven days of consuming treated wastewater irrigated-produce all Group I members exhibited quantifiable levels of carbamazepine, while in Group II the distribution remained unchanged from baseline (between group $P < 0.001$). Area under the curve of carbamazepine excretion was markedly higher in Group I versus II ($P < 0.0001$). Urine levels return to baseline following the exposure periods. This study demonstrates "proof of concept" that human exposure to pharmaceuticals occurs through ingestion of commercially available treated wastewater-irrigated produce, providing data which could guide policy and risk assessments.

92 Composting studies in Estonia

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This work is addressed to the application of biosolids and especially sewage sludge as a major resource in composting. Safe disposal of sewage sludge is one of the major environmental concerns. A considerable drop in the use of P fertilisers can be followed since early 1990s and due to this fact crop production in Estonia takes place largely at the expense of soil P resources. One of the ways of increasing the fertility of agricultural lands is to use nutrient-rich sewage sludge. Unfortunately, this may cause several undesired consequences due to biological and chemical contaminants present in this matter. The presence of some widely used pharmaceuticals, as ciprofloxacin (CIP), norfloxacin (NOR), ofloxacin (OFL), sulfadimethoxine (SDM) and sulfamethoxazole (SMX), was evident in sewage sludge of the two Estonian largest cities, Tartu and Tallinn. The concentrations of pharmaceuticals decreased significantly after sewage sludge digestion and composting (in most experiments their concentrations decreased by 95% or more during 4 months of composting), but they were still present in detectable amounts. Sewage sludge co-composting experiments with sawdust, peat and straw showed the degradation of FQs and SAs. Additions of sawdust clearly speeded up this process, whereas the mixtures with peat and straw performed lower abilities to decompose pharmaceutical residues. The presence of pharmaceuticals in our study affected the microbial community composition of the compost that resulted in lower microbial activity values and affected fungal-to-bacterial ratios in composts. In the end of the experiment the fungi dominated over bacteria in composts with added pharmaceuticals (60-70%). In compost where pharmaceutical residues were not added bacteria dominated over fungi (65%). Novel methodologies were developed and experiments conducted to study the potential accumulation of fluoroquinolones (FQ) and sulfonamides (SA) by food plants – taken up from the soil fertilized with sewage sludge or its compost. It has been shown that due to the low adsorption of SAs on soil particles they are "free" to migrate into plants. Different behaviour is characteristic to FQs as they are accumulated in sludge. Recent years have also shown certain progress in vermicomposting work, in studies involving microbial activity change during composting, and in using compost in afforestation.

93 Pentachlorophenol, chlorinated dioxins and furans in soil surrounding utility poles on the Kenai National Wildlife Refuge in Alaska

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A local electric utility company maintains above-ground transmission lines within the Kenai National Wildlife Refuge under U.S. Fish and Wildlife Service-issued right-of-way permits. Most supporting poles have been treated with commercial pentachlorophenol mixtures known to contain trace amounts of chlorinated dioxins and furans. These contaminants can migrate from treated poles to surrounding soils, posing potential risks to human health and ecological receptors. To characterize risk and ensure best management practices for pole replacement and/or removal, we collected surface soil samples near twelve utility poles for measurement of pentachlorophenol, chlorinated dioxin and furan congeners, and selected polycyclic aromatic hydrocarbons. There were six sets of paired poles; each pair consisted of a pole installed in the 1950s and a pole installed within the past twenty years that were located near each other. A representative background sample was collected for each pair. Samples were collected at 0 cm, 25 cm and 50 cm from each pole. Pentachlorophenol, dioxin and furan concentrations in soil were quite high at the base of each pole, and diminished approximately an order of magnitude from 0 cm to 25 cm, and again from 25 cm to 50 cm out from the pole. When expressed in terms of 2,3,7,8-tetrachlorodibenzo-p-dioxin toxicity equivalents, the average soil concentrations were 15.2, 5.17 and 1.52 parts per billion at 0, 25 cm and 50 cm distances from the pole, respectively (dry weight). Pentachlorophenol concentrations were 1800, 160, and 18 parts per million at zero, 25 cm and 50 cm distances from the pole, respectively (dry weight). Contaminant levels near the poles are orders of magnitude above screening levels for risk to both human and ecological receptors, warranting a more comprehensive risk assessment. This issue presents complex challenges for state environmental regulators, public land managers, and utility providers who strive to manage risk and protect human health and the environment, while continuing to provide electricity to Alaskan communities at a reasonable cost.

94 North of 60: Linking Herbicide Persistence to Soil Ecological Risk Along Transmission Right-of-Ways in the Yukon Territory

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Mechanical techniques, including brushing and mowing, are currently the only control methods used on transmission right-of-ways (ROWs) in the Yukon Territory. Primary issues with mechanical methods include increased density and regrowth of target species. Long-term vegetation control along transmission ROWs in the Yukon Territory may be improved through incorporating different management practices. These practices could integrate mechanical and chemical vegetation management with regional or site-specific techniques, such as ecological manipulation. Specifically, incorporating herbicides into the vegetation management program could improve long-term effectiveness, reduce environmental risk and decrease management costs. Therefore, obtaining a greater understanding of the impact of herbicides on the soil from the perspective of a northern terrestrial ecosystem is key in making informed vegetation management decisions. The persistence and toxicity of Garlon® XRT (triclopyr) and Arsenal® Powerline (imazapyr) in soils was assessed at five ROW locations representative of the main ecoregion types where ROWs occur within the Yukon Territory. Soils from four sites were collected at 1, 30 and 365 days after treatment (DAT) to determine persistence of herbicides for each of three application methods (backpack spraying, cut stump and point injection). Soils from a fifth site were collected as soon as herbicides had dried and at 1, 3, 7, 14, 21, 30 and 60 DAT. Increased sampling intervals were added in order to better determine the half-life of each herbicide in Yukon Territory soils. Herbicide residues are being extracted using traditional methods which vary for each herbicide. Results from the persistence testing will be linked to a species sensitivity distribution (SSD) created by a series of dose response tests, including three soil invertebrates, two non-target plant species and four soil enzymes, in reference soils from each field site. Preliminary results suggest that herbicide residues persist longer in northern regions than in temperate regions, however, these residues are below the protective herbicide concentration, or the concentration that impacts less than 25% of the soil community.

95 Evaluation of the environmental fate and ecotoxicological impact of the pesticide chlorpyrifos in soil for improvement of its risk assessment

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Pesticides protect crops from various pests but can also harm non-target organisms. To minimize risks for the environment and human health, a huge amount of studies are carried out and evaluated during the authorization process of each pesticide. However, the prediction of the environmental fate and ecotoxicological impact of a pesticide remains difficult. Several 100 formerly used pesticides are now banned because unexpected risks emerged decades after their authorization. Risk assessment documents of the organophosphate insecticide chlorpyrifos (CHL) particularly lack information about its degradation and potential transformation products in soil, and its impact on non-target microorganisms supporting soil ecosystem services with impact at the global scale. Within the frame of a large European Marie Curie project, we studied the environmental fate of CHL in a lab-to-field dissipation study by (i) quantifying CHL and its main transformation product 3,5,6-trichloropyridinol (TCP), (ii) screening for known and new transformation products, and (iii) measuring its sorption to soil. Furthermore, the ecotoxicological impact of CHL on the soil bacterial community was estimated by (i) Illumina next-generation sequencing of 16S rDNA amplified from extracted soil DNA, and (ii) monitoring the adaptation of soil microorganisms to repeated CHL exposure by analysis of CHL mineralization. Main results are (i) the identification of known and one new transformation products of CHL, (ii) no effect of CHL on the diversity of the bacterial community in our lab-to-field experiment, (iii) but adaptation to repeated CHL exposure as shown by improved CHL mineralization and by microbial diversity analysis. For further research on CHL biodegradation and as potential candidates for bioremediation, we isolated a CHL-degrading bacterial consortium.

96 So the Soil is Contaminated...Now What?

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The environmental risk assessment process in Ontario has become an exercise in exposure control as opposed to facilitating truly scientific, statistically-based risk analyses, environmentally sound decisions, or smart risk management. Risk-adverse provincial policies, misplaced authority, and the booming condominium industry have driven out science and the opportunity for learned practitioners to make sound judgements, particularly with regards to the management of contaminated soils and protection of ecological health. Moreover, efforts to ensure fully unbiased decision-making have resulted in an onerous administrative process that has nothing to do with environmental protection, but makes claims to that effect. This presentation will outline several "flaws" in the execution of Ontario Record of Site Condition (RSC) Regulation 153/04 from the perspective of how the mandated risk assessment process in Ontario differs from the practice as it is taught by academics, and endorsed by professional toxicologists and the SETAC membership.

Environmental Application of Cell-Based and High-Content Screening Assays for Monitoring Program and Risk Assessment

97 In vitro endocrine activity screening of waste and river waters from the Brussels Region, Belgium using the BG1Luc4E2 CALUX Bioassay

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Presence of endocrine disrupting or active chemicals in waterways is an ever growing global problem as evidenced by the WFD (2013/39/EU) who prioritized some model estrogenic chemicals into their watch list. Analyzing these substances typically involves separation chromatography coupled to mass detection at high cost per sample. However, performing a first screening of water samples using in vitro assays that detect endocrine selective ligands can help prioritize monitoring sites at lower cost. We therefore studied a highly impacted and urbanized river (Zenne River, Brussels, Belgium) that is composed of up to 50% in WWTP effluent downstream. We collected river water and influent and effluent of 2 major WWTPs (North and South) discharging to the river and a hospital effluent in order to gain more knowledge on their fate upon reaching this urban river. To accomplish this we collected water samples on a monthly basis during 1 year, performed SPE clean-up on the dissolved water phase, and analyzed these extracts using the BG1Luc4E2 in vitro transactivational bioassay to detect endocrine agonist activity. Concentrations of activity (BEQs) are reported as mass E2-equivalent concentrations. One of the most interesting features observed is that activity levels in the river tend to increase along the flow of the river, ranging from 1.34ng E2-eq./L to 3.80ng E2-eq./L at the most downstream site. Effluent levels of the two WWTPs (1.47 and 2.55ng E2-eq./L) displayed similar activity, but are able to drive activity variations depending on the river flow and their relative contribution there to. Influent values of the WWTPs and hospital ranged 1 to 2 orders of magnitude higher and pose real concern when untreated wastewaters are released into the river during combined sewer overflow events, which occur frequently in this water system and urban area. Furthermore, by implementing this monitoring program we obtained (annual averaged) in vitro activity results that surpass the limits proposed by the EU WFD (0.4ng/L for E2 and 0.035ng/L for EE2). Keeping in mind there is no clear-cut extrapolation or correlation between these effect-directed analyses and the chemically based limits, our results indicate this river system is highly impacted with endocrine activity and the responsible agents need characterization.

98 Combining cell-based bioassays, chemical analysis and mixture modelling for water quality monitoring

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Surface waters and drinking water may be impacted by a wide variety of organic contaminants, including pesticides, pharmaceuticals, industrial compounds and by-products of treatment processes. Cell-based bioassays have been applied for many years to complement chemical analysis for water quality monitoring, but similar to chemical analysis where the selection of target compounds remains a point of discussion, so is the choice of the appropriate biological endpoints and bioassays. Reporter gene assays are popular because they target selected individual nuclear receptors or transcription factors and can be adapted to a well reproducible, high-throughput screening format. The endpoints chosen should be anchored in the framework of the adverse outcome pathways and include several steps of the cellular toxicity pathways, including xenobiotic metabolism, molecular initiating events, adaptive stress responses and cytotoxicity. Here we present a strategy to select appropriate endpoints and apply them to several case studies focusing on the application of these tools for water quality assessment in wastewater, surface water and drinking water. Case studies were drawn from both the European Union project SOLUTIONS, which aims to produce solutions for legacy, present and future chemicals that pose a risk to both ecosystems and human health in European water resources, and from international efforts to harmonise the application of bioanalytical tools for evaluation of water recycling and reuse. Mixture toxicity modelling using the bioanalytical equivalent approach was applied to better link the results of the cell-based bioassays with chemical analysis. The case studies showed that for most endpoints the detected chemicals could only explain a small proportion of the effect, which supports the application of bioanalytical tools complementary to chemical analysis for water quality monitoring.

99 Application of the Attagene FACTORIAL™ assay to characterization of surface waters from a nationwide assessment of streams

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Bioassays can be used to evaluate the integrated effects of complex mixtures from both known and unidentified contaminants present in environmental samples. However, such bio-monitoring approaches have typically focused only on one or a few pathways (e.g. estrogen receptor, androgen receptor) despite the fact that the chemicals in a mixture may exhibit a range of biological activities. High-throughput screening approaches that can rapidly assess samples for a broad diversity of biological activities offer a means to provide a more comprehensive characterization of complex mixtures. The Attagene Factorial™ platform is a high-throughput, cell based assay utilized by USEPA's ToxCast Program, which provides high-content assessment of over 90 different gene regulatory pathways and all 48 human nuclear receptors (NRs). This assay has previously been used in a preliminary screening of surface water extracts from sites across the Great Lakes. In the current study, surface waters samples from 38 sites were collected, extracted, and screened through the Factorial assay as part of a USGS nationwide stream assessment. All samples were evaluated in a six point, 3-fold dilution series and analyzed using the ToxCast Data Pipeline (TCPL) to generate dose-response curves and corresponding half-maximal activity concentration (AC50) estimates. A total of 27 assay endpoints responded to extracts from one or more sites, with up to 14 assays active for a single extract. The four sites in four different states with the greatest number of active endpoints, ranging from 10 to 14, all were associated with anthropogenic wastewater inputs. Across assay endpoints, pregnane X response element (PXRE), aryl hydrocarbon (Ahr), and estrogen response element (ERE) were the most commonly activated endpoints, at 38, 33, and 25 sites, respectively. The results highlight the applicability of cell based, high-throughput assays, specifically the Attagene Factorial assay, to screening level prioritization of environmental sites or of biological pathways that may be adversely impacted. The contents of this abstract neither constitute, nor necessarily reflect, official USEPA policy.

100 Application of ToxCast to Evaluate Potential Biological Effects from Organic Contaminants in Great Lakes Tributaries

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With the development of "high throughput" in vitro biological assays, screening-level information on potential adverse biological effects is available for a rapidly increasing number of chemicals. The USEPA ToxCast program has now evaluated several thousand chemicals with more than 800 assays. The original intent of this data was to evaluate potential for human health effects, but it is now being extended to evaluate potential for environmental health effects given that ToxCast is based on evaluation of endpoints that are not necessarily organism dependent. The R software package ToxEval was developed as a screening tool to use ToxCast results for evaluation of potential adverse biological effects from trace organic chemicals in water samples. Using ToxEval, trace organic chemical data from water samples and passive samplers collected at 57 Great Lakes tributaries from 2010-2013 were examined to determine which tributaries had the greatest potential for adverse biological effects, with prioritization of the most influential contaminants. Results are being used as part of the

Great Lakes Restoration Initiative to focus current and future investigations that will help understand likely adverse outcome pathways in organisms, and to formulate possible remediation strategies.

101 Use of cell-based receptor assays to screen for endocrine disrupting chemicals and infer potential toxicity in California waters

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In vitro cell assays are being considered as bioanalytical screening tools for water quality assessment. Recent studies have shown that they can be used to benchmark water of different qualities, suggesting they can provide valuable information in monitoring programs to identify sites requiring further chemical and toxicity analyses. In this study, commercially available cell assays previously optimized for water quality screening were applied to detect endocrine disrupting chemicals (e.g. estrogens and glucocorticoids) in various aqueous samples from California (USA). Results revealed that secondary treated wastewater effluents had the highest levels of endocrine activity while the majority of freshwater streams analyzed showed little to no bioactivity. To further evaluate the potential of bioscreening cell assays to predict toxicity, a study was conducted to characterize the relationship between in vitro and in vivo responses induced by estrogenic chemicals. Larvae and juveniles of the inland silverside, Menidia beryllina were exposed to two estrogenic chemicals and biological endpoints including growth and gene expression changes were evaluated. Our findings indicate that in vivo effects occur at concentrations higher than in vitro responses, thus highlighting the potential of cell assays as screening tools for both occurrence and adverse effects of chemicals in the environment.

102 The Pro-Inflammatory Properties of Sediment Extracts from a Creosote-Contaminated EPA Superfund Site: Inflammation as a Form of Immunotoxicity

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The southern branch of the Elizabeth River, near Portsmouth Virginia, USA, is one of the most polluted sub-estuaries in North America, and home to the well-studied Atlantic Wood (AW) EPA superfund site. Prior to closure in 2014, this site was heavily contaminated with creosote, metals, and hydrocarbons. The chemical profile and developmental toxicity of sediment waters, as aqueous extracts, from the AW site has been extensively studied for over 20 years by other labs using Fundulus heteroclitus (Atlantic killifish). Based on decades of experimental research using a variety of mammals and fish, many of the contaminants found at the AW site are predicted to be highly carcinogenic and immunotoxic. Through past collaborations with others, we showed that adult AW killifish collected in situ had severe hepatic lesions, including hepatoblastoma and hepatocellular carcinoma, as well as suppressed circulating antibody responses. We also showed that innate immune functions were higher in AW fish, including elevated expression of COX-2, suggesting a pro-inflammatory environment. To further determine the potential of AW matrices to modulate innate immune function(s), this study used the mouse macrophage cell line RAW264.7 as an in vitro model. This cell line is very well characterized and a common in vitro model system for understanding macrophage-related inflammation profiles in immunotoxicology and immunopharmacology. Compared to reference sediment extracts, filter-sterilized aqueous AW sediment extracts induced inducible-nitric oxide synthase (iNOS) and cyclo-oxygenase-2 (COX-2), as well as nitric oxide and IL-6 secretion, at levels higher than induced by standard lipopolysaccharide (LPS, endotoxin) treatments alone. This enhanced pro-inflammatory property of AW extracts is due, in large part, to high levels of endotoxin(s)s (or similar TLR ligands), as polymyxin-B ameliorates this effect. Finally, using a commercially available limulus lysate assay, we show that bacterial endotoxin levels are significantly higher in AW extracts compared to reference extracts. These data suggest that such high induction of pro-inflammatory activity by contaminated sediment extracts is an expected form of toxicity that should be considered in future environmental immunotoxicology studies.

103 Assessment of Potential Environmental and Health Impact of Coal Ash Spill in Dan River, North Carolina

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On Feb 2, 2014, the third-largest coal ash spill of U.S. occurred at Eden, N.C., spewing large amount of coal ash and contaminated water into the Dan River. After the spill, parameters such as TOC and trace elements were monitored by USEPA and Duke Energy, and showed elevated trace elements such as As, Cd, Se and Sr in river water, sediment and soil. The concerns of short and long term impacts of the spill demand investigation of fate and transport of toxic substances released from the ash, as well as the associated toxicity and implied ecological and health risks. This project aims to provide a fast and initial toxicity assessment of Dan River water and sediment with spatial and temporal resolution, using a quantitative toxicogenomics-based in vitro assay platform in both yeast and human cells. Toxicity related to genotoxicity, oxidative stress, chemical stress, protein damage and apoptosis were evaluated using proteomics or transcriptional assays. The toxicity profiles allowed for integrated multivariate statistical tools to reveal temporal and spatial patterns, and the relationship with chemical properties. Surface water and sediment samples from eight sites up- and downstream of the spill at three time points over five months were assessed. Compared to control location upstream of the spill, there seemed to be elevated toxicity in the middle part of the river downstream of the spill, especially for Anglers Park and Milton. Similar trend was also observed for trace elements, with relatively high concentration of As at Anglers Park. Toxicity associated with chemical stress (chemical transport, membrane and cell wall) and general stress (apoptosis, cell signaling and metabolism) were significantly enriched, which was consistent with expected effects of trace elements such as As and Pb. Statistical and correlation analysis, as well as “iceberg” metal mixtures confirmed that that the toxicity detected by yeast library was mainly contributed by the detected trace elements. Dissolved organic matters characterized by TOC and fluorescence spectroscopy seemed to only contribute small portion of the toxicity effects detected in this study. The results suggested potential ecotoxicological implications of trace elements in the coal ash. This study demonstrated the cost-effective toxicogenomics-based in vitro assay platform as an effective toxicity method for timely and informative water quality monitoring, as urgently needed by incidents like N.C. coal ash spill.

104 Quantitative Toxicogenomics Assay Revealed the Impact of Disinfection Technologies On Effluent Toxicity

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The current water and wastewater treatment effectiveness assessment that focuses on targeted contaminants concentration measurements alone is insufficient because it cannot detect the complex risks resulted from mixtures of many contaminants and transformation products (TPs) in the water. This study applied a novel, fast, and cost-effective quantitative toxicogenomics-based approach for evaluation of the toxicity evolution of wastewater effluents disinfected by different technologies, including chlorination, chloramination, ozonation, UV as well as combination of UV and H_2O_2. The impact on overall toxicity, measured by Microtox test and toxicogenmics assay, varied among different disinfection technologies. The toxicogenomics approach showed consistent yet more sensitive results than Microtox test, therefore resulted less sample enrichment. Moreover, the toxicogenomics assay revealed technology-specific toxicity profiles, indicating distinct nature among disinfection technologies and associated specific mixtures of TPs. Chlorination and ozonation showed the highest toxicity reduction efficiency while chloramination is the worst that actually led to significant increase in the effluent toxicity. Although

UV treatments showed no significantly effects on overall toxicity reduction, the toxicity profiles exhibited unique patterns specific to UV that suggested the presence of DNA-damaging TPs. Combination of UV with H_2O_2 seemed to remove the DNA-damaging substances that generated by UV-treatment alone. To further evaluate the contributions of targeted CECs residues to the overall effluent toxicity, we conducted iceberg experiment that compared the toxicity of artificial mixture of 26 CECs detected in the effluent to those of real effluents. The results showed that for most samples, the targeted CECs in the effluents likely only explain part of the whole water toxicity, indicating that the production of toxic TPs. In addition, it highlights the insufficiency of water quality monitoring based on targeted contaminants concentration measurements alone. In summary, this study demonstrated that toxicogenomics-based assay could not only help to evaluate the impact of technology on overall effluent toxicity, but also reveal potential causal agents and toxicity evolution nature. In company with chemical analysis, this quantitative toxicogenomics approach can help to provide guidance on technology optimization, implementation for effluent risk reduction.

Integrating Life Cycle, Risk and Alternatives Assessment with Exposure Modeling for Chemical Decision-Making

105 Impact of oil spill on a palm oil plantation: a Life Cycle Assessment (LCA) study

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Ecosystems could be wild or managed and in both cases they can serve as sources of food, recreation and even employment. The above then suggests that, ecosystems are prerequisite for health and life. In poor countries, especially in rural areas, the health of rural communities may directly depend on locally productive ecosystems as oil palm plantation by providing sources of some forms of livelihood. An ecosystem could negatively be impacted by pollution as in the case of an oil spill incident that affected Owaza oil palm plantation, Abia State, Nigeria. The Owaza local community's dependent on this plantation (ecosystem) for livelihood is apparent but very fundamental. This paper offers a Life Cycle Assessment (LCA) methodology which hinges on valued ecosystem components (VECs) as suitable indicators for impact assessment of the oil spill incident that affected Owaza oil palm plantation. In this work we considered a goal function of ecosystems which is to maximize the dissipation of ecosystem productivity by maximizing the biological functions of the biotic components of the ecosystem such as communities of plants and animal species found within. These functions include photosynthesis by green plants, biological nitrogen fixation (BNF) by diazotrophic bacteria, and aeration of soil by burrowing activity of earthworms. Crude oil spill impact may decrease ecosystem productivity by decreasing chlorophyll level or inhibiting the sunlight energy-harvesting capacity of chloroplast, reduction in fixed soil nitrogen/nutrient cycling and mortality rate/burrowing activity of earthworms. Within this theoretical framework, an assessment of the impact of crude oil spill in Owaza oil palm plantation was carried out by measuring the capacity of the palm trees (in terms of chlorophyll content) to trap light energy. The technique of measuring solar energy trapping potential of chlorophyll (the palm trees), coupled with impediment on VECs was considered a promising tool. Once operational, it could offer a quick and cheap alternative to evaluate crude oil spill impacts in any terrestrial ecosystem of any size. Integration of the above into an ISO compatible LCA framework may be explored.

106 Joint Uncertainty and Sensitivity Analyses for improved and aligned LCA and RA

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Several works have signaled the need of integrating the life cycle assessment (LCA) and risk assessment (RA) methodologies, due to several common aspects. In this contribution, we show that a powerful way to reach the integration between LCA and RA is through guaranteeing that the output uncertainty and the input parameters influence on the output uncertainty are carefully studied, and communicated. Even though the topic has been attracting increasing attention of practitioners and experts, there is still a lack of understanding and a limited use of the available statistical tools. In this contribution, we introduce a Monte-Carlo protocol to jointly conduct uncertainty and global sensitivity analyses for both LCA and RA models. We focus on a newly developed multi-media fate and transport model, CLiCC-o. Fate and transport models are at the basis of the impact assessment phase of LCA, but are also directly used to characterize human and ecological safety in RA of chemicals. The output of CLiCC-o can be, in fact, directly compared with risk factors, or, alternatively, connected to an effect and exposure model to calculate characterization factors for LCA impact assessment. We use CLiCC-o as a test case. CLiCC-o has 89 environmental parameters and 12 chemical properties as inputs, and is further defined for 22 compartments (e.g. air, freshwater, marine water) and 6 geographical archetypes (e.g. San Francisco Bay). The outputs of the model are daily concentrations in the 22 compartments and geographies. The model inputs are correlated, the model is highly nonlinear, and highly multiplicative. We show that jointly tackling uncertainty and sensitivity is fundamental to guaranteeing that the modeler has a complete understanding of: (1) the uncertainty of the model outputs (2) the structure of the model and (3) the importance of uncertain model inputs and the interaction among them. The combined use of LCA and RA techniques may further be fostered by such a platform. The protocol helps setting rules and a common shared procedure that puts the uncertainty and sensitivity analyses practice in the field of LCA in line with the practices that are more common in RA community, thus de facto improving the value of the information that the tools provide to decision-makers.

107 What do LCA, Decision Analysis, and Community Resilience have in common? Beyond the Chemical Exposure Models

C. Stahl, USEPA Region III

This presentation will discuss two related topics around the concept of doing multi-criteria environmental assessments that include non-chemical stressors as a step toward nurturing community resilience. These topics are: 1) The importance of distinguishing science from non-science components in LCA and decision analysis, and 2) psychosocial effects as an example of a non-chemical stressor that could be made part of a quantitative assessment. Both of these will be illustrated using a case example, using a first principles-directed approach, the Multi-criteria Integrated Resource Assessment (MIRA). What do we want to assess? How to assess the environmental condition of an area subject to mining or drilling? What are the factors that go into answering these questions and are they all purely scientific? Likely not. Much of what is actually missing isn't science but how we use the science, a subjective but (hopefully) not arbitrary decision. Making this distinction between what is science and what is not increases our capability to seek more appropriate approaches to fill in gaps. Do we run an exposure model, build a new model or tool, conduct an expert elicitation or better understand stakeholder needs and values? As we talk about gap-filling, what are the data for non-chemical stressors, such as psychosocial effects? How can we avoid committing the sin of measuring what we can instead of measuring what is important; and how can we avoid perpetuating this into our decision making? Communities often name psychosocial effects as some of their most important concerns and yet, statutory and regulatory requirements, Agencies, and exposure models do not address them. Aside from the discomfort of considering a non-chemical stressor that is viewed as amorphous, untestable, or subjective, excuses about lack of studies or data are often used to justify excluding these kinds of stressors. However, if we change how we think about indicators, it is possible to think quantitatively about qualitative things. We talk broadly about seeking to achieve resilience. However, achieving resilience is not limited to those parameters that are described by the outputs from chemical exposure models. Resilience isn't just for the hippie tree-huggers. Learn how your technical and scientific knowledge can be part of the solution set.

108 Integrating risk metrics into Life Cycle Assessment using rapid exposure modeling for consumer products: Air freshener case study

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A case study was performed to explore methods to integrate high-throughput risk-based screening into life cycle assessment (LCA) for consumer products. It has been recognized that combining LCA and risk assessment (RA) can lead to more comprehensive assessments. LCA evaluates multiple impacts such as climate change, land use, and human health impacts associated with a product, system, or service. Single chemical human health RA (HHRA) evaluates the probability of adverse response associated with human exposures to a given chemical. One difference between LCA- and RA-based exposure calculations is that in LCA calculations are conducted based on a functional unit of the product being studied and in RA dose estimates are often based on exposure to all sources of a chemical. One interesting application of the combination of LCA and HHRA is the analysis of consumer products, to account for environmental sustainability metrics for a product alongside human health risks associated with exposures to chemicals in the product. Exposures to chemicals associated with consumer products can be substantially larger via indoor and near-person pathways (near-field) compared to exposures resulting from releases to the outdoors. This implies that for many chemical-product combinations there may be a higher probability of risk from near-field chemical sources compared to chemicals released outdoors. Methods to prioritize and screen chemicals for risk are becoming available and are based on high-throughput exposure modeling and screening toxicity data; however these methods have yet to be incorporated into LCA. 1,4-dichlorobenzene (p-DCB) in an air freshener was used as the chemical-product combination in the case study. LCA human health impact scores were calculated for p-DCB based on typical household use of an air freshener. High-throughput risk-based screening was based on use of p-DCB in all relevant product types. Both methods indicated that exposure to p-DCB was dominated by near-field exposure pathways by at least two orders of magnitude. The risk-based screening also suggested that exposure to p-DCB was dominated by consumer product sources other than the air freshener. This case study can be used to inform the methodological integration of LCA with risk-based screening methods and to further investigate how results from risk metrics can be incorporated into the interpretation of LCA results taking into account other environmental impacts.

109 Human health risk evaluation from chemical exposure in an LCA context – ProScale

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Increasingly, various stakeholders require information on eco/toxicity aspects of products beyond regulatory requirements for chemicals, e.g. in the framework of Life Cycle Assessment ("LCA"). There is currently no methodology available which fully satisfies the requirements of the industry and other stakeholders in this context, but requirements are often based on lists for priority substances without evaluating their risk in specific applications. This calls for a performance based indicator that can be used and communicated within LCA's, Environmental Product Declarations ("EPD's") and Product Environmental Footprints ("PEF's"). The work presented here is performed in an ongoing project, ProScale, for developing a method for comparing hazard and exposure potential to chemicals constituents of products in a life cycle perspective. The primary intended use is a performance indicator to be provided as additional information in LCA and particularly in PEF and EPD according to the international standards ISO 14025 and EN15804 alongside other LCA impact categories. The ProScale partners are: BASF, Covestro, Deutsche Bauchemie, DSM, IVL, Kingspan, Solvay. The project runs in its current phase until end of 2016. The method developed aims to be a science-based, transparent, pragmatic and generally applicable methodology for a toxicological exposure assessment of products, and should be characterized by: (i) assess the relevant direct exposure potential along the whole life cycle; (ii) use existing data, e.g. REACH based; (iii) allow comparison in relation to technical performance; and (iv) be relevant for business-to-business and business-to-customer communication.In brief, the method is starting from the product ingredients and combines hazard factors and exposure factors for each substance and life cycle stage. The chosen approach for the hazard factor is based on H-phrases, as these are readily available. An ongoing challenge is to refine this approach through the application of a potency factor based on, e.g., derived no-effect levels (DNEL) or occupational exposure limits (OEL). The chosen approach for the exposure factor is to make use of exposure modelling tools available, such as ECEtocTRA or similar for occupational exposure and ConsExpo for consumer exposure. An ongoing challenge here is to safeguard that different modelling tools used for different exposure give compatible results. Initial results based on case studies will be presented.

110 Elements from hazard, exposure, and life-cycle to inform an integrated approach for assessing alternatives

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The community working to assess alternative ingredients and products face a variety of challenges to balance sometimes competing goals including 1) reduction of the risk associated with chemical ingredients; 2) rewarding chemicals and products that are designed using the principles of green engineering and chemistry; 3) viewing multiple impacts across the spectrum of life cycle stages, all the while 4) maintaining functional and economic performance. Overall, any alternative chemical or product must perform similarly or better than its predecessor, be measurably safer for the user and the environment, and have an economic profile comparable to the chemical or product it replaces. Full alternative assessments as currently practiced involve a substantial amount of expert resources, data, and time so it is important that there is a screening approach to focus resources on viable candidates. Given the broad spectrum of functional roles chemicals and products play, there is currently no “best” solution that will work in all circumstances. This work suggests a set of concepts and elements useful for a screening-level evaluation that directly responds to the question or problem and provides relevant information to the decision at hand. The goal is to suggest conceptual elements and approaches to relate risks with life cycle environmental impacts and to discuss the challenges and possibilities when working toward a process for integrated decision-making. A series of case studies or examples will be used to illustrate the concepts.

111 Integrating Exposure into Chemical Alternatives Assessment Using a Qualitative Approach

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Most alternatives assessments (AA) published to date are largely hazard-based rankings, and as such may not represent a fully informed consideration of the advantages and disadvantages of possible alternatives. With an assessment goal of identifying an alternative chemical that is more sustainable, other attributes beyond hazard are also important, including exposure, risk, life-cycle impacts, performance, cost, and social responsibility. Building on the 2014 recommendations by the U.S. National Academy of Sciences to improve AA decisions by including comparative exposure assessment, the HESI Sustainable Chemical Alternatives Technical Committee, which consists of scientists from academia, industry, government, and NGOs, has developed a qualitative comparative exposure approach. Conducting such a comparison can screen for alternatives that are expected to have a higher human or environmental exposure potential, which could trigger a higher-tiered, more quantitative exposure assessment on the alternatives being considered, minimizing the likelihood of regrettable substitution. This talk will demonstrate an approach for including chemical- and product-related exposure information in a qualitative AA comparison. Starting from existing hazard AAs, a series of three chemical-product application scenarios were examined to test the concept, to understand the effort required, and to determine the value of exposure data in AA decision-making. The group has developed a classification approach for ingredient and product parameters to support comparisons between alternatives as well as methodology to address exposure parameter relevance and data quality. The ingredient parameters include a range of physicochemical properties that can impact routes and magnitude of exposure, while the product parameters include aspects such as exposure pathways, use pattern, frequency/duration of use, chemical concentration in product, and use volume, accessibility, and disposal. Key learnings, challenges, and opportunities for further work will also be presented. The views expressed in this presentation do not necessarily reflect the views or policies of the U.S. Environmental Protection Agency.

112 Hazard and Exposure Assessment Screening in Alternatives Assessment

H. Plugge, 3E Company

Establishing functionally equivalence criteria for a chemical/product of concern is a first step in alternatives assessment. Whether de novo designed or selected from the library of existing chemicals, all alternatives will need to undergo a hazard assessment and exposure assessment screening phase. Different hazard assessment methodologies provide apples and oranges as effects making it very hard to compare overall impacts from different alternatives. The presentation will demonstrate a hybrid scientific data/classification approach to hazard screening /assessment, using a composite scoring system for each individual aspect of health effects. Screening of health effects using this methodology would allow the non-expert to derive a preliminary hazard screening. One of the outcomes of this screening would be the identification of datagaps and data anomalies. A near simultaneous step would be the performance of an exposure screening where preliminary simplified exposure assessment data are derived allowing the calculation of a relative risk screening. Again the units here are consistent in a simplified assessment such as this, allowing easy summation for each alternative. More user input would be required here to allow the establishment of realistic exposure scenarios. Preliminary life cycle considerations can also be reviewed at this stage, although not required under the new TSCA regulations. This risk (= exposure * hazard) screening process only weeds out the really much worse alternatives. In addition significant datagaps will be identified. Hence a feedback cycle with screening is established – as datagaps are filled (using Read Across and QSAR among others) , the screening assessment will become more accurate. The number of alternatives which are about equal (or better) than the initial chemical/product, will hopefully be whittled down to about three. A detailed assessment is then to follow using additional data analytics and data gap filling techniques to derive as complete a database as possible with the available resources.

New and Persistent Contaminants in the Environment from the Reuse of WWTP Effluent Streams

113 Identification of Emerging Contaminants from a Waste Water influenced water body Using High Resolution Accurate Mass LC/MS and Statistical Analysis

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Historically pristine sources of water are under increasing threat of contamination from a plethora of point and non-point discharges. With over 15,000 new chemicals introduced daily it is impractical and economically unfeasible to monitor each one. Non-targeted methods using high resolution accurate mass MS can identify many compounds, but the contents of these discharges are not often available in databases. Determination of what components are significant and focused identification of these compounds are critical, but difficult given the variability of environmental samples from the same sampling points at different times. This work examines LC/HRMS data of two creeks in Pennsylvania that feed into the Susquehanna river which is the drinking water source for millions of Americans in the north east. The creeks are impacted by agricultural runoff, fracking and a wastewater plant discharge and were monitored over 6 months using multivariate statistics to determine compounds that should be monitored. 6 creek samples, a sample of the wastewater effluent discharging into the creek, filed and lab blanks were collected and then analyzed using automated online solid phase extraction and an LC-QTOF/MS. Initial Non-targeted analysis using the accurate mass pseudo-molecular ions and fragments of real LC peaks co-eluting against the custom database gave positive identification including pharmaceuticals, pesticides, personal-care products and others using databases and libraries. A statistical analysis of the samples was performed to find different features in each sample. PCA of the statistically significant compounds showed that the wastewater effluent replicates formed a tight group well separated in the first principle component from all other groups. The creek sample downstream was slightly separated from the final effluent but well separated from the upstream creek samples. All blanks formed a tight group well separated from the others. The PCA analysis demonstrates that the statistical procedures can distinguish compounds found at the different points of collection. The non-targeted database analysis also demonstrates that although compounds of interest can be determined, there are many more compounds that cannot using a limited database and statistical analysis will assist in identifying relevant and ‘emerging’ contaminants to be monitored in the future.

114 Wastewater Recycling in Antarctica – Challenge of Micro-contaminant Assessment by Passive Sampling

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The Australian Antarctic Division (AAD) operates Australia's Davis station in the Antarctic and has decided to install a new advanced water treatment plant (AWTP) in conjunction with a secondary wastewater plant to ensure discharges to the environment meet world's best practice. The AWTP was tested during 2014-16 using water from a municipal wastewater treatment plant in Tasmania to see whether it was capable of producing potable water and a non-toxic brine concentrate that can be discharged with minimal environmental impact. The process requirements of the AWTP are small (~20 kL/day) and although the inputs to the plant are source defined because of the known community size and chemical manifests, without the dilution achieved in large scale WWTPs there is potential for spikes in chemical contaminants. One of the challenges for the AWTP is to adequately meet the risks associated with chemicals (single chemicals and mixtures) in its product water. One option is to use passive sampling. In 2015-16, we deployed Chemcatcher passive samplers in the Feed Water, RO concentrate (environmental discharge) and Final Product Water (recycled water). The passive samplers were deployed for one month, with water sampling on the day of deployment and retrieval the following month when a new passive sampler was deployed. This process was undertaken for consecutive months in the autumn of 2015, and then again for 2 months in the winter of 2016. Each passive sampler replicate contained either an Empore™ SDB-XC or an Empore™ C18FF disk, respectively, covered by a polyethersulphone (PES) or Omnipore rate limiting membrane (to stop the receiving phase becoming saturated too quickly). Two multi-residue chromatographic-mass spectrometric methods and a range of recombinant receptor-reporter gene bioassays were used to screen trace organic chemicals (TrOCs), toxicity and receptor activity of the water and passive sampler extracts. This paper will discuss the potential environmental and human health risks of the chemicals observed in water and passive sampler extracts, and the suitability of passive sampling for monitoring the performance of the AWTP in Antarctica.

115 Interactions between dissolved organic matter, organic pollutants and carbon nanotubes in the environment

M. Engel, The Hebrew Univ of Jerusalem; B. Chefetz, The Hebrew Univ of Jerusalem / Soil and Water Sciences

In recent years, herbicides and pharmaceuticals have been frequently detected in aquatic environmental systems. Their presence poses a major threat and therefore efforts must be invested in expanding our knowledge on the behavior of such pollutants in the aquatic environment and in exploring prospective applications for their removal. Carbon nanotubes (CNTs) have drawn special attention in light of their superior adsorptive capabilities. Furthermore, their increasing use will inevitably lead to their release into environmental systems where they may interact with organic pollutants and dissolved organic matter (DOM). Adsorption of organic compounds by CNTs has been widely examined, but the effects of DOM on adsorption under different solution conditions have been rarely addressed. Hence our main objective was to research the influence of DOM on adsorption of the herbicide atrazine and anticonvulsant drug lamotrigine by CNTs in varying solution conditions and introduction stages of DOM. This study shows that atrazine and lamotrigine compete over the same adsorption sites. However atrazine was a far more effective competitor than lamotrigine, suggesting there are adsorption sites preferentially occupied by atrazine. The presence of DOM greatly suppressed adsorption of both pollutants. For example, in the midst of their adsorption isotherms, adsorption of atrazine decreased by 94%, and that of lamotrigine by 69%, at high concentration of DOM, respectively. This may be attributed to competition or blockage of adsorption sites by DOM. However, decrease in adsorption of lamotrigine may also be allocated to the formation of a stable DOM-lamotrigine complex with lower affinity to the CNTs. Increased stability of lamotrigine (and not atrazine) was demonstrated by its enhanced solubility in DOM solution (solubility of lamotrigine was increased by up to 70% in DOM solution). When the CNTs were first exposed to DOM, adsorption of both pollutants was greatly suppressed. But when the pollutant was given the initial advantage, its' adsorption still decreased after DOM was added to solution. This finding implies there are DOM molecules with higher affinity to the CNTs which can replace the adsorbed pollutant and force its' release back into solution. Our findings will advance the understanding of interactions between organic compounds and CNTs in environmental systems containing DOM and improve the potential employment of CNTs in organic pollutant removal technologies.

116 Occurrence and Mass Balance of Fipronil & its Degradates in Wastewater and Biosolids of Eight Wastewater Treatment Plants in Northern California

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Fipronil is a phenylpyrazole insecticide used primarily in urban areas for outdoor and subterranean pest control and pet flea treatment. Recently it has been shown to adversely affect sensitive non-target aquatic invertebrate species at low part-per-trillion concentrations (< 100 ng/L), and also has been identified as a chemical of concern in the global collapse of honeybee colonies. The objectives of this study were to (i) conduct a mass balance assessment during activated sludge treatment; (ii) evaluate the occurrence and fate of fipronil and four of its major degradates during treatment, at eight wastewater treatment plants (WWTPs) of Northern California sampled under low-flow drought conditions. Analytes were determined by liquid chromatography tandem mass spectrometry (LC-MS/MS). Mass balance was conducted at a conventional WWTP located in southwestern U.S. and based on flow-weighted samples collected on five consecutive days. Aqueous phase total fipronil appeared to pass through treatment, as mean daily loads of total fipronil related compounds in aqueous phase of raw sewage versus treated effluent ($p = 0.29$) were virtually unchanged. In the eight studied WWTPs, fipronil and its sulfone and sulfide derivatives were detected with 100% frequency in all influent and effluent samples, with concentrations ranging between 13-88 ng/L, 1-28 ng/L, and 1-5 ng/L, respectively. Fipronil amide, a product of fipronil hydrolysis, was absent in all influent samples but detectable in effluent of seven of the plants at concentrations ranging between 1-4 ng/L, suggesting hydrolysis during biological treatment. When taking into account the mass of fiproles associated with particulate matter, a small fraction of the total fiprole loading (35 ±11%) partitioned into sludge and thus was removed but not degraded during wastewater treatment. Overall, 65 ±11% of the total mass of fipronil related compounds entering the plants remained were discharged in treated effluent to surface waters. Concentrations of fipronil compounds detected in sludge ranged between 0.1-91 μg/kg dry weight. The ratio of sulfone and sulfide degradates to fipronil was higher in biosolids when compared to raw wastewater. Results suggest that none of the treatment processes examined in this work provide significant fiprole degradation, resulting in unwanted insecticide presence in both treated effluent and sludge.

117 Kinetics of carbamazepine uptake, transformation, and metabolism in cucumber plants and its drug-drug interaction with lamotrigine

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Carbamazepine is a common drug used for the treatment of seizures in epilepsy patients. Carbamazepine is found at low mg/L levels in many sources of drinking water, and in fresh water and treated wastewater used for crop irrigation. Its nonionic state and log Kow of 2.45 make it easy to cross membranes, and to be translocated in the plant predominantly with the transpiration stream, thus accumulating mostly in the leaves. In past studies, we have demonstrated the uptake, translocation, accumulation and metabolism of carbamazepine in several cropsin different soils irrigated with treated wastewater. A number of carbamazepine metabolites are known to be as pharmacologically active as the parent compound and in some cases may be of toxic nature, thus highlighting the need to follow metabolism of pharmaceutical compounds in plants. In this study a timeline of the uptake and translocation of carbamazepine and the formation of several of its metabolites were established over a time period of 96 hours for cucumber plants grown under hydroponic culture. Drug-drug interaction with lamotrigine, also used in the treatment of epilepsy, was evaluated. A linear increase in carbamazepine concentration in both roots and shoots was found and the rate of uptake was shown to be at a lower rate than water uptake, indicating its permeability is lower than reported in past publications. As well as the parent compound, we were able to identify and quantify several of its metabolites. The main metabolites found were 10,11-epoxide-carbamazepine (which is a reactive and pharmacologically active compound), 10,11-dihydroxy-carbamazepine, 2-hydroxy-carbamazepine and 3-hydroxy-carbamazepine. These are also the main products of carbamazepine metabolism in the human liver. Enhanced root metabolism was identified between 72-96 hours pronounced by an elevated 10,11-epoxide-carbamazepine concentration in the plant sap. In the presence of lamotrigine, lower concentrations of 10,11-epoxide-carbamazepine were found in the plant material, indicating decreased metabolism caused by drug-drug interaction. This data is of great importance for further understanding plant uptake and metabolism of xenobiotics – their formation and accumulation in different plant compartments.

118 Comparison of the relative efficacy of two cost effective sampling approaches for fingerprinting VOC emissions from land-disposed sewage sludge wastes

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About 8 million dry tons of sewage sludge is generated annually in the US, with well over half now disposed on agricultural and forested lands as soil amendments known euphemistically as "Biosolids." Though containing beneficial nutrients for plant growth, sludge also contains complex mixtures of volatile organic compounds (VOCs) that generate odoriferous and toxic emissions. Indeed, we have observed first hand the stifling odors, as well as acute toxicity of large-scale sludge disposal to resident macrobiota of the sensitive rainforests of the Puget Sound watershed. Though ecotoxic impacts are a primary concern, it is the repugnant odors that are most obvious to and disconcerting for the public and which have escalated concerns for the safety of this practice. Measuring volatile emissions from sludge in situ is challenging, typically requiring complex and costly apparatus. To address this problem, we hypothesized that simple low-density polyethylene plastic strips could be deployed at sludge disposal sites to characterize associated VOC emissions for minimal cost and sampling effort. To evaluate their potential for this, we conducted a controlled headspace exposure assay to assess the capacity for these simple devices to effectively sample sludge-associated VOCs and provide diagnostic fingerprints thereof. For comparison, we also evaluated the efficacy of simple headspace sampling using gas tight syringes. While both approaches revealed emissions of complex VOC mixtures (e.g. hydrocarbons, halogenated hydrocarbons, phenols, ethers, amines, organosulfides, plasticizers and personal care product constituents, etc.) in the ppb to ppm range, each yielded distinct VOC fingerprints. These findings demonstrate the prospective utility of these very simple sampling approaches to provide low cost/low effort diagnostic fingerprints of VOC emissions from land-disposed sewage sludge.

119 Behavior of polyfluorinated alkyl substances, including PFOS and PFOA, in wastewater systems: Experiences from multiple studies in North America

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Poly- and per- fluorinated alkyl substances (PFAS) including perfluorooctanoate (PFOA) and perfluorooctanesulfonate (PFOS) are ubiquitous in the environment due their persistence, and the widespread historical and continuing use of a variety of fluorinated chemicals in applications ranging from fire fighting, mist suppression, food packaging, stain and water resistance application in clothing, and more. Analytical and regulatory attention has focused primarily on PFOS and PFOA, and more recently, on other per- and polyfluorinated compounds in groundwater and other contamination associated with fluorinated compound manufacture, AFFF sites, and industrial sites. WWTP plants also have significant loadings of PFAS, including a wide variety of more complex polyfluorinated compounds that may transform into perfluorinated carboxylic acids (PFCAs) and perfluorinated sulfonic acids (PFSAs) that can be more persistent in the environment than the precursors. These transformations have been observed in both ambient conditions, and in wastewater treatment plants. Therefore, understanding the fate, transformation and mass balance of PFAS through the wastewater treatment process is critical. Current research indicates an increase in PFCAs and PFSAs through conventional wastewater treatment processes, and potential for formation of PFCAs and PFSAs from a wide variety of chemistries, including fluorotelomer alcohol and sulfonate based products. In this work, PFAS occurrence and behavior from multiple studies in WWTP processes in North America is summarized. All analytical work was carried out using isotope dilution/surrogate standard quantitation LC-MS/MS techniques that provided recovery correction and accounting for matrix effects. This enabled robust cross- compartment and inter-study comparisons, partitioning calculations, mass balance studies and more. The analysis of WWTP components presented specific analyte challenges, primarily matrix suppression, which was identified and compensated for using isotope dilution and other analytical techniques.

120 Comparing the environmental input of emerging contaminants from conventional and alternative WWTPs: a targeted and suspect screening study

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Wastewaters are a well-defined source of emerging contaminants into the environment. Conventional, tertiary wastewater treatment systems do not effectively remove many emerging contaminants, including pharmaceuticals and perfluorinated compounds, resulting in the discharge of chemical contaminants into waterways. Forest-water reuse systems are alternative wastewater treatment systems that infiltrate municipal, industrial, and agricultural wastewater through forest soils via slow-rate irrigation. Forest-water reuse systems provide ecosystem service benefits and are lower cost and energy than conventional treatment systems and have recently been investigated as to their pharmaceutical input. However, to better understand their role as a source of emerging contaminants, these systems need to be directly compared to conventional systems. In this study, both a quantitative, targeted and qualitative, suspect screening approach were utilized at a forest-water reuse system and conventional tertiary wastewater treatment system to better understand how emerging contaminant input from a forest-water reuse system compares to a conventional system. Quantitatively, greater concentrations and total mass flow of pharmaceuticals was exhibited downstream of the conventional treatment system. From a suspect screening standpoint, more confirmed chemicals were present, and at a greater relative abundance, downstream of the conventional system as well. This data shows that forest-water reuse systems can reduce the environmental input of emerging contaminants to a greater degree than conventional systems.

Chemical, Biological and Instrumental Methods for Detecting Harmful Algae and Their Natural Toxins

121 Prevalence of cyanotoxin microcystin-LR in sediments in California's Stream Pollution Trends (SPoT) Program and potential toxicity to invertebrates

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Cyanobacterial harmful algal blooms (CHABs) are an emerging threat to drinking water resources. Microcystis cyanobacteria can release hepatotoxins which affect diverse taxa. Microcystins are stable cyclic heptapeptides that may persist in the environment for weeks to months in water and sediments. Most monitoring efforts have focused exclusively on water samples. California's Stream Pollution Trends (SPoT) Program monitors 100 base of the watershed sites to assess long term trends in toxicity, contaminants and land use. In collaboration with California State University Monterey Bay, SPoT has identified microcystin-LR presence in sediments monitored statewide using modified extraction methods and quantified using enzyme-linked immunosorbent assay (ELISA). Microcystin-LR was detected in 31% (n = 108) of the samples in 2014 and 13% (n = 96) in 2015. Microcystins were found in sediments from diverse habitat types and land uses. In both years, a subset of sites were analyzed in an inter-laboratory method validation study, using ELISA as well as LC-MS. By evaluating sediments at the base of major watersheds throughout the state, SPoT provides a baseline assessment for microcystin-LR prevalence and occurrence statewide. In order to assess the ecological implications of algal toxins in streambed sediments, studies are underway to determine the potential for chronic toxicity to aquatic invertebrates and bioaccumulation with10 d Microcystis exposures using the midge Chironomus dilutus. In addition, experiments evaluating toxicity of anatoxin-a from Phormidium sp. to the amphipod Hyalella azteca, midge C. dilutus, and cladocera Ceriodaphnia dubia will be presented and discussed.

122 Method Development, Monitoring, and Occurrence of Microcystins in Ambient Water

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The occurrence and intensity of cyanobacterial harmful blooms have become increasingly common over the last few decades. Cyanobacteria are a worldwide concern in areas with eutrophic water conditions. Cyanotoxins generated from cyanobacteria are harmful ecologically, cause economic impact, and are a public health threat. The accurate detection of harmful cyanotoxins has become increasingly important in the protection of human and ecological health. The USEPA has taken steps to improve the analytical methodology available for the detection and quantification of cyanotoxins. Specific steps include developing methods for cyanotoxins in drinking and ambient water and providing standardized detection and analysis methods for emerging cyanotoxins of concern. EPA recently developed Method 544 for determination of microcystins (MCs) in drinking water using solid phase extraction (SPE) and liquid chromatography/tandem mass spectrometry (LC/MS/MS). Method 544 has been modified for use in ambient water. A review of these methods will be presented with a summary of the challenges associated with method development and modifications for ambient water analysis. A monitoring study for cyanotoxins was conducted in a local Ohio lake in 2015 and again in the summer of 2016. Total MC (extracellular + intracellular toxin) concentrations for thirteen MC congeners at several sites within the lake were measured. A summary of MC concentrations in water samples will be presented, including congener type and the frequency of detection. A comparison of concentrations measured by LC/MS/MS and enzyme-linked immunosorbent assay (ELISA) will be presented, along with a comparison of data from the 2015 and 2016 sampling seasons. In a separate but related study, water samples will also be collected from various lakes or rivers around the United States with moderate to severe cyanobacteria blooms. Data will be presented from these samples for MC concentration, congener profile, and comparison to ELISA concentration.

123 Comparative measurement of microcystins in diverse surface waters using ADDA-ELISA, LC-MS/MS, and MMPB techniques

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The measurement of microcystins, cyanotoxins associated with cyanobacterial blooms which are increasingly prevalent in inland waters, is complicated by the diversity of congeners which have been observed in the environment. At present, more than 150 microcystin congeners have been identified, and this poses a significant challenge to analytical methods intended to assess human health risks in surface and drinking water systems. The most widely employed analytical method at present is the ADDA-ELISA technique which is potentially sensitive to all microcystins, but it is primarily intended as a semi-quantitative method, and questions have been raised regarding the potential for cross-reactivity and false positives. LC-MS/MS methods targeting specific congeners, such as USEPA Method 544, are intended for use as a secondary confirmation following a positive ELISA response, but these techniques can target only those congeners for which commercial standards are available. Accordingly, they are not suitable for ascertaining the safety of a given water sample, given the potential for omitting unknown microcystin congeners which may be present. An alternative approach involves oxidative transformation of microcystins to a common product, 2-methyl-3-methoxy-4-phenylbutyric acid, or MMPB. Measuring MMPB by LC-MS/MS can potentially provide a metric for the sum of all microcystin congeners present in a sample, subject to the efficiency and overall yield of conversion. The present study discusses the results from the application of the MMPB method, applied in conjunction with ADDA-ELISA and individual congener measurement using LC-MS/MS, to measure microcystins in bloom-impacted surface water samples obtained from multiple sites across the United States. Results of toxin measurements will be compared between sites and analytical techniques, and optimized conditions for the MMPB procedure will be presented.

124 Cyanotoxin occurrence associated with cyanoHAB events on an inland reservoir

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A monitoring approach combining wet chemistry and high frequency (HF) water quality sensors has been employed to improve our understanding of the ecology of an inland reservoir with a history of cyanoHAB events. Lake Harsha is a multi-use reservoir managed by the USACE in southwest OH that has experienced an increase in cyanoHAB frequency and intensity. Nutrient, algal taxa, chlorophyll-a, and physico-chemical data have been collected on the lake since 2010 at three week intervals. Beginning in 2014 in cooperation with Clermont County Drinking Water Division, a high frequency monitoring program was implemented to complement the tri-weekly data and provide DWTP operators time-relevant information regarding source water quality. High frequency data included in vivo fluorescence and physico-chemical parameters and were collected at two locations. These data, coupled with microcystin (MC) analyses demonstrated the utility of HF data for tracking the cyanoHAB status of the reservoir. It was also apparent that MC concentrations were potentially underestimated as MC sampling did not coincide with bloom peaks indicated by the HF data. To better characterize the cyanobacterial population and both intracellular and extracellular MC production, an intensive sampling regime was implemented in 2015 and 2016 including LC-MSMS analysis of select MCs, cylindrospermopsin, and anatoxin-a, MC ELISA quantification, molecular analyses, inhibition assay, nutrients, chlorophyll-a, and total organic carbon. Samples were collected weekly except during the initial bloom in June, when samples were collected daily. This approach allowed for the characterization of the cyanobacterial population dynamic and greatest periods of MC production. Data and observations for the period 2014-2016 will be presented.

125 New fluorometer uses the parameter 'unbound phycocyanin' as an early warning system for cyanobacterial T&O compounds and cyanotoxins

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New type of fluorometer uses the parameter 'free PC' ('unbound phycocyanin') as an early warning system for the appearance of difficult to treat cyanobacterial T&O compounds and cyanotoxins The concept of a new spectral fluorometer is demonstrated. This instrument is designed to estimate the potential risk of the appearance of T&O compounds resp. cyanotoxins from cyanobacteria in raw and drinking water online. Goal of the research was to investigate the correlation between the appearance of Geosmin, 2-MIB and/or other taste and odor (T&O) compounds from cyanobacteria, any cyanotoxins by the detection of the presence of 'free phycocyanin'. 'Free phycocyanin' describes that part of the cyanobacteria marker pigment 'phycocyanin' which is not energetically bound to chlorophyll anymore due to lysis and cellular stress. The disrupted phycocyanin has might still be intracellular in a lysed cell with affected membranes but often has already left the cell (extracellular). This lysis can be caused by senescence, mechanically strongly treated raw water or oxidants resp. disinfectants in drinking water treatment plants. Oxidants and disinfectants will affect the cell walls and the cell lysis starts delayed after a certain period of time. T&O compounds the free PC will leave the cells accordingly at the same time; the free PC can be used as an indicator. The method has proven its potentials in the lab already applying mechanical treatment and oxidants to cyanobacteria cells. The estimation of risk described here depends on the cyanobacteria concentration in raw water, its physiological state and the effects of the treatment steps in the water works.

126 Harmful algal bloom smart device application: using image analysis and machine learning techniques for early classification of harmful algal blooms

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Reports of toxic cyanobacterial blooms, also known as Harmful Algal Blooms (HABS) have increased drastically in recent years. HABS impact human health from causing mild allergies to liver damage and death. The Ecological Stewardship Institute (ESI) at Northern Kentucky University is developing a smart device application that will permit accurate and quick identification of potential HABS. This new application, titled HAB APP, will be used to assist identification of HABS in recreational and drinking water supplies. Using support vector machine computer learning algorithms, the smart device extracts a color histogram from an image and compares it with a pre-loaded trained model of images for classification. More specifically, the algorithm distinguishes between relatively harmless green and potentially harmful blue-green algae. The algorithm will be extended to classify images taken from cameras situated at a nearby lake and along the Ohio River and will include other classes of algae.

127 Effects of eutrophication in fish from Brazilian water supply reservoir

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Reservoirs are water bodies that are used to power energy, water supply and recreation. These anthropogenic activities have caused several problems and environmental impacts in the aquatic ecosystems, which are associated with the eutrophication. Eutrophication results in algae and cyanobacteria blooms. Iraí Reservoir is used to water supply and is located in southern Brazil. The aim of this study was to monitor the water quality in the Iraí Reservoir, the potential toxic effects and depuration of cyanotoxins on Geophagus brasiliensis. Water samples and fish samples were collected in Iraí Reservoir from September 2015 to May 2016. Water samples were used to qualitative and quantitative analyses of phytoplankton and quantification analyses of cyanotoxins. Fish were divided in two groups. The first one was called "site group", which were euthanized after been sampled. The second group was called "depuration group", which was submitted to depuration experiment for 90 days. This experiment was divided in 7, 15, 30 and 90 days. Blood samples were taken to comet assay and liver and kidney tissues were removed in order to measure effects using biomarkers such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione S-transferase (GST), glutathione reduced (GSH), lipoperoxidation (LPO), metallothionein (MT) and comet assay. Phytoplankton samples showed dominance of Microcystis aeruginosa e Dolichospermum planctonicum, both toxic cyanobacteria. Cyanotoxins quantification is being analyzed. Our results suggested recovery of the antioxidant system and reduction of cellular and DNA damage. GPx and GSH activities increased when lipid damage decreased, indicating a protective effect. Likewise the DNA damage in the blood decreased in 90th day, but the liver damage increased in 15th and 30th day, and it was recovery in 90th day. This result suggested biotransformation in metabolites that can cause damage. Furthermore, in the 30th day the GST activity increased. According to our multifactor and ordination analysis, the depuration starts in 15th day of the experiment and the set of the biomarkers was effective to show the differences among the groups. Therefore, aquatic organisms are exposed to contaminants mixture in Iraí Reservoir and the monitoring of water quality in this area is needed to minimize the risks to the human health and to the aquatic environment.

128 Analysis and Occurrence of Marine Biotoxins in the Catalan Coast of Spain at the Western Mediterranean Sea

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Marine biotoxins are secondary metabolites synthetized by phytoplankton and bacteria. The toxic properties of these compounds have a negative influence in the growth, reproduction and survival of certain species. Moreover, they can bioaccumulate along the food chain[1]. Therefore, their presence is an issue of concern, especially when harmful algal bloom (HAB) takes place. The objective of this study is to assess the occurrence and distribution of 18 toxins pertaining to 4 groups of marine biotoxins in the coastal waters of Catalonia, Spain. The toxins selected for this study were: Azaspiroacids (azaspiracid-1, AZA1; azaspiracid-2, AZA2; azaspiracid-3, AZA3; azaspiracid-4, AZA4; and azaspiracid-5, AZA5); and related pectenotoxins (pectenotoxin2, PTX2), yessotoxins (yessotoxin, YTX; and homoyessotoxin (hYTX); okadaic acid (OA) and related dinophysistoxins (dinophysistoxin-1, DTX1); domoic acid, DA; saxitoxins (neosaxitoxin, NEO) and related gonyautoxins (gonyautoxin-2, GTX2; gonayutoxin-3, GTX3; gonyautoxin-5, GTX5; decarbamoylgonyautoxin-2, dcGTX2; decarbamoylgonyautoxin-3; dcGTX3; N-sulfocarbamoylgonyautoxin-2, C1; N-sulfocarbamoylgonyautoxin-3, C2); and tetrodotoxin, TTX. Therefore, the first objective was to develop and validate a multiresidue analytical method based on solid phase extraction (SPE) followed by High performance liquid chromatography coupled to high-resolution mass spectrometry (HPLC-HRMS) using a Q-Exactive (Thermo Fisher Scientific) hybrid quadrupole–Orbitrap mass spectrometer with an electrospray ionization source operated in positive and negative modes. The method detection limits were in the range of 0.52 to 0.14 µg/l, exhibiting good reproducibility and linearity. Afterwards, surface seawater samples collected at 20 different locations along the Catalan coast (NE of Spain) were analyzed. In each sampling site, water samples were collected inside and outside marinas. Okadaic acid was the prevalent compound found in 90% of the samples witn concentrations ranging from 2.10 to 1778.10 ng/l. Acknowledgement: This work was supported by the by the European Union through the projects Sea-on-a-chip (FP7-OCEAN-2013-614168) and Bravoo (FP7-OCEAN-2013-614010).

21st Century Approaches for Cross-Species Extrapolation in Toxicity Assessment

129 A Cell-Free Neurochemical Assay to Screen Chemicals across Fish, Birds, and Mammals

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An estimated 30,000+ commercial chemicals are believed to possess neurotoxic properties. As current animal studies are expensive, time-consuming, and overlook many at-risk organisms, development of new screening methods is required. In vitro, cell free assays have potential as relatively inexpensive, screening mechanisms to flag neurotoxic compounds. The objective of this study was to advance a cell-free, neurochemical-based screening assay platform, and to apply it to study 20 species of birds, fish, and mammals, and screen against ~900 chemicals. In Phase 1 of this project, we screened 80 known environmental chemicals (metals, pesticides, personal care products, flame-retardants) against key receptors and enzymes from the glutamatergic, GABAergic, dopaminergic, and cholinergic neurochemical pathways, and did so in multiple taxa including fish (king mackerel, yellowfin tuna, goldfish, rainbow trout, perch), birds (bald eagle, Japanese quail, chicken, zebra finch), mammals (river otter, mink, pilot whale, common dolphin, narwhal, ringed seal, polar bear), and biomedical species (rat, mouse, human). In Phase 2 of this project we took a sub-set of Phase 1 “hits” and derived concentration-response curves. In Phase 3 of this project, we focused on a subset of species and neurochemical endpoints, and screened the USEPA E1K library of 800 chemicals. In this presentation, in addition to presenting our results we will 1) elaborate on the execution of such cell free assays and discuss ‘pros and cons’ of our screening method; and 2) discuss a strategy by which large volumes of in vitro data outputs may be modeled to predict individual-based adverse outcomes pathways.

130 Cross-species extrapolation of uptake and disposition of organic chemicals in fish using a multi-species physiologically based toxicokinetic model

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The potential to bioaccumulate is generally considered an unwanted substance property. Consequently, chemical legislations, such as the European REACH regulation, require the chemical industry to provide bioconcentration data for chemicals that are produced or imported at volumes exceeding 100 tons per annuum, or if there is concern that a substance is persistent, bioaccumulative and toxic. To fill the existing data gap for chemicals below this stipulated volume without the need for additional animal experiments, physiologically based toxicokinetic (PBTK) models can be used to kinetically predict whole-body bioconcentration as well as internal concentrations of neutral organic chemicals in different fish tissues. PBTK models have been developed for many different fish species with promising results. In this study, we developed PBTK models for zebrafish (Danio rerio) and roach (Rutilus rutilus) and combined them with existing models for rainbow trout (Onchorhynchus mykiss), lake trout (Salvelinus namaycush) and fathead minnow (Pimephales promelas). The resulting multi-species model allows for cross-species extrapolation of the bioaccumulation potential of neutral organic compounds. Predictions were compared with experimental data and were accurate for most substances. Our model holds strong potential for the probabilistic risk assessment of a chemical’s bioaccumulation potential with special emphasis on cross-species extrapolation of sensitivity.

131 Applying phylogenetic methods to interspecific toxicity extrapolations

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The tremendous diversity of chemicals used in commerce coupled with the tremendous biodiversity of life on Earth guarantees that we will always be data deficient with respect to understanding chemical hazards to biota. Recent investment in high-throughput (and reductionist) approaches to understanding toxicity is further diverting limited resources from whole animal toxicity evaluation. Thus, the need for rational methods for interspecific toxicity extrapolation is substantial. The manifestation of toxicity is a complex trait resulting from a combination of subordinate traits related to absorption, distribution, metabolism and excretion (ADME) and target site sensitivity. The analysis of species traits in other disciplines (e.g. evolutionary biology, ecology) has demonstrated that the tendency for related species to share common traits is ubiquitous. The development of statistical approaches based on the phylogenetic relatedness of species allows us to quantify the relationship between phylogeny and traits of interest. In this talk, we present an overview of these phylogenetic approaches and efforts by our group to apply them to subordinate traits related to trace metal absorption and excretion vary across faunal groups. We further examine variation in carbaryl toxicity across faunal groups, demonstrating strong phylogenetic linkages with toxicity. Together, these results show proof-of-concept that phylogenetic approaches can be fruitful in interspecific toxicity extrapolations.

132 Improving toxicity extrapolation using molecular sequence similarity: A case study of pyrethroids and the sodium ion channel

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A significant challenge in ecotoxicology has been determining chemical hazards to species with limited or no toxicity data. Currently, extrapolation tools like USEPA’s Web-based Interspecies Correlation Estimation (Web-ICE; www3.epa.gov/webice) models categorize toxicity estimation between the surrogate and predicted species by broad taxonomic groups. Toxicity predictions between more distantly related taxa have greater uncertainty, thus identifying variations in sensitivity across taxonomic levels may improve extrapolation. Because species sensitivity is a function of phylogenetic relatedness, understanding conservation of a chemical’s molecular target can likely inform cross-species predictions of sensitivity based on whole organism toxicity data. EPA’s Sequence Alignment to Predict Across Species Susceptibility (SeqAPASS; https://seqapass.epa.gov/seqapass/) tool can be used to determine cross-species molecular target similarity by comparing protein sequences to a known susceptible query species. The goal of this work was to explore the relationship between sequence similarity of key functional domains of the voltage-gated sodium channel, derived from SeqAPASS, and curated, mode of action specific, acute toxicity data for pyrethroids to improve correlation based toxicity extrapolation. Sequence similarity was determined at the species, genus, and family level to identify variability in sensitivity at each taxonomic level. These analyses showed that species sensitivity to pyrethroids was correlated with the sodium channel gate and ion transport domains across taxa. These results will be used to improve cross-taxa toxicity extrapolation models and reduce uncertainty in predictions by grouping species with similar molecular target sequences and chemicals with the same molecular initiating event.

133 Comparison of toxicological and transcriptional pathway point of departure responses for five chemicals across five divergent species

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Many efforts are underway to replace animal testing with in vitro assays or animal test models (e.g. fish embryos, Daphnia magna, Caenorhabditis elegans, or human cell lines). To better understand conservation of toxicological response across divergent species, we exposed C. elegans, Daphnia magna, zebrafish embryos and liver differentiaed human pluripotent stem cells to chemicals known to impact specific targets [flusilazole and hexahydro-1,3,5-trinitro-s-triazine (RDX)], an endocrine disruptor (bisphenol A), and a thyroid disrupter (perfluorooctanesulfonic acid), and a liver and hemoglobin toxicant [2,4,6-trinitrotoluene (TNT)]. In our comparison synchronized stage L3 C. elegans were exposed for 24 hr to a range of chemical concentrations, 48h old D. magna were exposed in aqueous media for 48h, 6 hr post fertilization zebrafish embryos exposed for 96hr, and differentiated liver cells were exposed for 24hrs. Mortality, morphological, and behavioral endpoints were monitored where applicable. We also examined changes in transcriptomes in response to chemical exposure in each species. Using transcriptomic data, we identified dose responsive pathways consistent with modes of action pathways and Adverse Outcome Pathways (AOP) known to be associated with each chemical. We then developed points of departure representing genes in those pathways to compare conservation of genes and pathways to toxicological responses across species. In general, pathway based point of departures occurred at lower concentrations than apical effects in all species. Across species, both pathways and toxicological sensitivity were more highly conserved within phylogenetically close alternative animal models. The data suggests that using transcriptional pathway based approaches can inform applicability and extrapolation of toxicological data from one species to another, thereby supporting better development of animal testing models.

134 Predictivity of the ToxCast ER model to non-mammalian in vivo reproductive endpoints measured in environmentally relevant species

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The USEPA's Endocrine Disruptor Screening Program (EDSP) has been charged with screening thousands of chemicals for their potential to affect the endocrine systems of humans and wildlife. In vitro high throughput screening (HTS) assays have been proposed as a way to prioritize chemicals for EDSP Tier 1 screening. A recently developed computational network model of the estrogen receptor (ER) pathway integrated 18 in vitro HTS ER assays within the ToxCast research program and made bioactivity predictions for 1812 chemicals. This computational model is strongly correlated with the in vivo rodent uterotrophic assay. This analysis was performed on 103 of the 1812 chemicals in the ToxCast ER model due to the availability of curated uterotrophic data. By contrast, the USEPA's ECOTOX knowledgebase contains 235 chemicals with population-level or vitellogenin effects with an overlap of 121 of the 1812 ToxCast ER model chemicals. This additional chemical coverage allows for additional assessment of model performance. However, it is unclear how the in vitro HTS assays, generated in mammalian cell- lines and mammalian receptors, correlate with in vivo effects in environmentally relevant species (e.g. fish). Previous work demonstrated ER model concordance with population-level and vitellogenin effects with sensitivities of 0.76 and 0.80, respectively. It should be noted that there were no entries for vitellogenin or population-level effects on mammalian species within ECOTOX. Given the species diversity within each reproductive category, the analysis was repeated considering each vertebrate group separately. Thus, the ER model had greatest sensitivities in predicting fish>amphibians>>birds for both population-level and vitellogenin effects. Test species was explored as a possible confounding factor. When fish vitellogenin data (48 species, 79 chemicals) was limited to only include standard fish experimental species (4 species, 67 chemicals), sensitivity increased from 0.80 to 0.86. This is likely due to a combination of data availability, experimental design, experimental reproducibility, and potential species variation in response. Given the high sensitivity between particular ecological endpoints and the current ER model, future work will look to incorporate existing ecological endpoint data into the ER model to improve and expand chemical prioritization and testing decisions.

135 In Vitro Activation of AHR2, but not AHR1, is Predictive of In Vivo Sensitivity to Dioxin Across Phylogenetically Diverse Species of Fish

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Adverse effects of exposure to dioxin-like compounds (DLCs) in vertebrates are primarily driven by activation of the aryl hydrocarbon receptor (AHR). However, mechanisms for the great differences in sensitivity to these effects among species of fish were unknown. Therefore, this study 1) investigated sensitivities to activation by the model DLC, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), of AHR1s and AHR2s among seven species of fish known to differ in sensitivity to TCDD by almost 40-fold, and 2) characterized the relationship between in vitro sensitivity to activation of AHRs to TCDD and in vivo sensitivity of embryos to TCDD. All AHR1s and AHR2s were activated in a concentration-dependent manner by exposure to TCDD. There was no significant linear relationship ($R^2 = 0.24$) between EC_{50} of AHR1 and LD_{50} of embryos. However, a highly significant positive linear relationship ($R^2 = 0.96$) was observed between EC_{50}s of AHR2s and LD_{50}s of embryos. The slope and y-intercept for this linear relationship for AHR2 of fishes is not statistically different from the slope and y-intercept for the previously determined significant linear relationship among EC_{50} of AHR1 and LD_{50} of embryos of birds to TCDD. Results of this study suggest that sensitivity to activation of AHR2, but not AHR1, mediates adverse effects of and sensitivity to TCDD among phylogenetically diverse species of fish with a comparable relationship as previously demonstrated for AHR1 of birds. This co-relationship resulted in a single equation for predicting sensitivity to TCDD across distantly related species of oviparous vertebrates from EC_{50}s of AHRs. This mechanism-based biological model has the potential to guide more objective ecological risk assessment of DLCs for species of fish that are not easily studied, including threatened or endangered species.

136 Development of an Adverse Outcome Pathway for Progesterone Receptor Agonism Leading to Reproductive Failure in Fish

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Environmental toxicology research involving pharmaceuticals has increased greatly over the last 10-15 years. Several of these compounds have been extensively researched due to their potential impacts to the endocrine system of aquatic organisms. The main focus of endocrine

disruption research has centered on how estrogenic and androgenic compounds interact with the endocrine system to elicit reproductive effects. Synthetic progestins have only recently been investigated in which these compounds display negative reproductive consequences in teleost species. Using a cross-species extrapolation approach, the development of an adverse outcome pathway (AOP) for progesterone receptor agonism and its relationship to reproductive failure in fish can be constructed. The use of progesterone receptor homology and mammalian receptor binding affinities of synthetic progestins aid in the prediction of negative impacts of synthetic progestins in fish. The goal of this presentation is to present both qualitative and quantitative data for the support of the development of an AOP for progesterone receptor agonism as it relates to reproductive impairments in fish.

Assessing Risks of Pesticides to Federally Listed (Threatened and Endangered) Species at a National Level – Part 2

137 Update on Methods Used to Estimate Aquatic Pesticide Exposure to Threatened and Endangered Species

C. Peck, C.M. Rossmeisl, J. Carleton, USEPA; T. Hawkes, National Marine Fisheries Service; R. DeWitt, NOAA; G.E. Noguchi, US Fish and Wildlife Service / Ecological Services

Since 2013, the Environmental Protection Agency (USEPA), the National Marine Fisheries Service (NMFS), the U.S. Fish and Wildlife Service (USFWS), and the U.S. Department of Agriculture (USDA) have been developing methods to evaluate exposure and risk on a national scale to aquatic threatened and endangered species resulting from pesticide applications. The methods were released for public review in April of 2016 with the publication of the draft Biological Evaluations (BEs) for three pilot chemicals (chlorpyrifos, diazinon, and malathion). Public comments were solicited and a stakeholder workshop was held to address some of the more complicated issues presented in the pilot BEs, such as exposure predictions in flowing waterbodies. This presentation will provide an overview of methods developed and updated by the Agencies for assessing aquatic species exposure in advance of the release of the final BEs for the pilot chemicals in December 2016.

138 Characterizing Pesticide Exposure to Threatened and Endangered Species with GIS

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The National Marine Fisheries Service, U.S. Fish and Wildlife Service, and U.S. Environmental Protection Agency (EPA), with input from the U.S. Dept of Agriculture (USDA), are developing a national framework for determining the effects of EPA registered pesticides on species and their critical habitats listed under the U.S. Endangered Species Act (ESA). Here we describe the process used to spatially characterize pesticide exposure to listed species and their designated critical habitats. We generate pesticide use-site footprint layers that represent application sites for agricultural and non-agricultural label uses. The Cropland Data Layer (produced by USDA) was used to produce 11 nationwide agricultural use layers. Additional data sets such as the National Land Cover Database (NLCD) were used to generate the non-agricultural use layers. The use-site layers were then expanded to incorporate the off-site transport of pesticides via runoff, drift, and downstream dilution. The resulting layers were then overlaid with species' range and designated critical habitat to determine where species' co-occur with each use. Knowledge of species life-history and distribution, as well as pesticide application rates, timing and methods are used to characterize species' spatial and temporal exposure within their range. Examples of this GIS-based exposure analysis will be presented by using the smalltooth sawfish (Pristis pectinate), listed as endangered in 2003, as a case study. The abundance of smalltooth sawfish in US waters has decreased dramatically over the past century (currently 5% of historical levels). This presentation will also explore some of the uncertainties inherent in the spatial data associated with species range, depiction of use sites, and pesticide exposure.

139 Refined pesticide exposure modeling for endangered species in flowing water habitats

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In their recent Biological Evaluations for three organophosphate insecticides, the USEPA concluded that improved pesticide exposure modeling approaches are needed for simulating pesticide exposure in flowing water habitat. To address this need, a species-specific, spatially distributed watershed scale exposure modeling approach at the 12-digit hydrologic unit code (HUC12) resolution was applied to evaluate chlorpyrifos exposure to flowing water habitats. Over 80,000 HUC12 watersheds cover the continental United States, including streams and rivers representing the low, medium, and high flow generic habitats proposed by the USEPA and the Services. The modeling approach combined the PRZM5 model, the traditional field scale landscape model used by the EPA, and the Soil and Water Assessment Tool (SWAT) watershed scale water quality model. The PRZM5 model was applied in a distributed manner to represent the variability in landscape and agronomic conditions within each HUC12 watershed. The parameterization of the PRZM5 simulations incorporated best available spatial datasets describing the land use and cropping patterns, soils, and weather. The timing of chlorpyrifos applications was based on regional agronomic practices and varied across watersheds, reflecting differences driven by field conditions, resource availability, and pest pressure. Conservative estimates of the percent of crops treated with chlorpyrifos were also included. The PRZM5 simulations provided surface runoff, erosion, and pesticide fluxes entering each HUC12 stream, while the SWAT model simulated realistic time-varying baseflow contributions to streamflow for each HUC12 watershed, providing a significant improvement to the assumption of constant baseflow. The channel system modules of SWAT simulated the downstream routing of the HUC12-level stream inputs. In-channel pesticide environmental fate processes were accounted for, including water and benthic degradation, equilibrium partitioning, settling and resuspension, and volatilization. The uncertainty in several parameters affecting chlorpyrifos environmental fate, baseflow, and channel routing were addressed by generating an ensemble of model simulations representing the expected range of these parameters. Probability distributions of chlorpyrifos exposures were derived for each species range and habitat type and can be used in combination with probabilistic effects determinations to inform risk characterization for the chemical.

140 Toxicity of Pesticide Mixtures in Midwest Streams

M. Shoda, USGS; W.W. Stone, USGS / Indiana Water Science Center; L. Nowell, USGS / California Water Science Center

In order to understand the vulnerabilities of streams in the Midwest and make decisions about areas that may need more intensive monitoring, an understanding of how pesticide mixtures occur in streams and their influence on biological communities is necessary. The Pesticide Toxicity Index (PTI) is a tool for assessing relative toxicity of pesticide mixtures to fish, benthic invertebrates and cladocera in stream water. It is a robust screening tool that incorporates the ability to assess multiple pesticides at once, which is how organisms encounter pesticides in a natural environment. The Watershed Regressions for Pesticides (WARP) models use multiple linear regression to identify watershed characteristics that are used as explanatory variables to predict concentration statistics for individual pesticides in streams. WARP methodology was used in this case to develop models using data from one hundred sites in the Midwest, sampled in May-August of 2013. The highest calculated PTI was modeled for these sites using watershed variables including toxicity-weighted agricultural use intensity, land use, agricultural management practices, soil properties, precipitation and hydrologic characteristics. A unique model to predict stream PTI was generated for fish, benthic invertebrates and cladocera. Toxicity-weighted use-intensity was an explanatory variable common to each of the models and the only variable that directly represents a pesticide source term. Some of the differences in significant explanatory variables between models as well as the differences in model performance may be related to the different pesticides that contributed the most to the highest calculated PTI values. The models were evaluated with independent data, not limited to the Midwest, and found to perform reasonably well with no clear bias. The WARP-PTI models for fish, benthic invertebrates and cladocera were also used to estimate the probability of exceeding a threshold PTI in Midwest streams. These models provide a low resource way to assess PTI at unmonitored streams in the Midwest and provide insight into how pesticide mixtures in streams influence biological communities.

141 Refining Co-occurrence and Proximity Analyses for Endangered Species Assessments

A.R. Frank, Compliance Services International

The FIFRA Endangered Species Task Force (FESTF) has contributed to the USEPA's endangered species and pesticide assessment process for nearly 20 years. Throughout this time, FESTF's efforts have been focused on aggregating, improving, analyzing, and providing data to the USEPA to inform their assessments. The recent release of USEPA's Draft Biological Evaluations, including national-level endangered species assessments, on the first three organophosphates through the registration review program, illustrated the need for improved and refined processes and methods that can enhance efficiency and accuracy while meeting species protection and agricultural goals. The FESTF has aggregated various data sets that can be used to develop a realistic portrayal of agricultural practices, their relationship to species presence, and the mitigating effect of certain environmental and species characteristics. This presentation will explore ways to refine and improve upon USEPA's Action Area definition, species ranges, and the resulting co-occurrence and proximity analyses.

142 Mitigation of Pesticide Drift from Aerial Applications by Riparian Vegetation

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In 2015, the Washington State Department of Agriculture conducted a targeted monitoring study to assess the effectiveness of riparian vegetation at reducing pesticide loading to streams during aerial applications of the organophosphate insecticide malathion. Applications took place at blueberry fields in Whatcom County, Washington. Two control sites (with no dense woody vegetation) were compared to three vegetated sites (with mature riparian vegetation). Vegetation was characterized by height, width, species diversity, and canopy cover. Site and channel geometry was also measured at each site. Eight total malathion application events were monitored at; four each at control and vegetated sites. Pesticide movement was monitored using depositional samplers at the field edge, the beginning of the riparian vegetation, and the center of the stream. Based on statistical modeling by Washington State University, instream malathion deposition was significantly lower at vegetated sites than control site. The site and vegetation characteristics that most reduced instream malathion deposition were increased distance from field (both to vegetation and to water) and increased canopy angle and canopy cover. This analysis predicted that an additional 26% reduction of instream malathion deposition could be achieved by either increasing the distance between the field and the beginning of the riparian vegetation by an additional 0.6 m or increasing canopy cover by an additional 9%. The benefits that riparian vegetation can have for habitat and water quality are already well known. This evidence that riparian vegetation is also effective at reducing drift into streams from aerial pesticide applications makes installation of more riparian buffer vegetation even more important.

143 Implementing an Integrated Pesticide Use Enforcement System in California

J. Haasbeek, CaliCo Solutions LLC; D. Marciano, California Dept of Pesticide Regulation; J. Gless, California Agricultural Commissioners and Sealers Association

The California Department of Pesticide Regulation (DPR) and the California Agricultural Commissioners and Sealers Association (CACASA) have been working together over the past several years to develop integrated management systems for restricted materials permitting, pesticide use reporting, and compliance and enforcement activity tracking. The most recent addition to the Pesticide Use Enforcement (PUE) program is a mobile inspection application that provides field inspectors with immediate access to data from numerous sources within the pesticide regulatory domain, including permitting, licensing, labeling, use reporting, and enforcement. This presentation will highlight the benefits gained from compiling information from these different programs and discuss the difficulties encountered and solutions found over the course of the project implementation.

144 Testing a "Net Conservation Benefit" Approach for Pesticides Using a Small-Scale Pilot for Listed Threatened and Endangered Species

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Enacted in 1973, the Endangered Species Act (ESA) is intended to protect animals and plants determined to be endangered or threatened with extinction. Courts have ruled that pesticide registrations conducted under the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) are actions subject to the ESA. The scale and scope of these assessments are daunting and unprecedented; under FIFRA, EPA Office of Pesticide Programs registers (permits) pesticides after review of the extensive scientific data set required by Congress, conducting comprehensive reviews of thousands of environmental and human health studies on more than 1000 active ingredients used in more than 19,000 formulated products that are used across broad geographies. EPA and the Services are currently attempting to chart a workable process for FIFRA consultations under ESA, which has been an overwhelming task given legal, scientific and procedural complexities. An approach is needed that reduces complexity, results in true landscape-level improvements for endangered species habitats and allows for a transparent and predictable approach to FIFRA/ESA consultations. Recent presidential memoranda direct federal agencies to incorporate into their decision-making the beneficial ecological services provided by landscape level conservation, and the use of conservation improvement as a mitigation option. Our goal is to conduct a small-scale pilot to test such a net conservation benefit program in this context. Our approach involves: Concept development through interaction with a wide array of experts on endangered species, habitat improvement programs, ESA and FIFRA law, government, farmers and other pesticide users. Selection of the agricultural pest-control practices, species and geographies of the pilot Development of a regulatory framework for demonstration during the pilot that will relate potential pesticide impacts (after avoidance and minimization measures inherent in FIFRA registrations) with on-the-ground habitat improvement work. Measures of successes and areas for improvement and adjustments to the pilot. The presentation describes the overall pilot approach and presents the status and results achieved as well as plans going forward.

Everglades and Wetlands Science Part 1: Ecology and Contaminants in Ever-Changing Ecosystems

145 Mercury contamination in sharks and the relationships between mercury concentrations in various shark tissues

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Mercury (Hg) exposure poses a threat to both fish and human health. Sharks are known to bioaccumulate Hg, however, little is known regarding how Hg is distributed between different tissue types (e.g. muscle regions, fins, liver, kidney, other organs). We evaluated total mercury (THg) concentrations from eight muscle regions, four fins, and five organs from two different shark species, bonnethead (Sphyrna tiburo) and silky shark (Carcharhinus falciformis) to determine the relationships of THg concentrations between and within tissue groups. THg concentrations were highest in the eight muscle regions with no significant differences in THg concentrations between the different muscle regions and muscle types (red and white). Significant relationships were found between THg concentrations in dorsal axial muscle tissue and the fin inner core, liver, kidney, spleen and heart for both species as well as the THg concentrations between the dorsal fin trailing margin and the heart for C. falciformis and all other sampled tissue types THg concentrations for S. tiburo. We then tested for THg concentrations in shark, ray, and fish meat (muscle) being sold in two open-air markets in San Jose, Costa Rica. THg concentrations were highest in Sphyrna zygaena (3.50 ± 0.47 ppm wet wt) followed by Carcharinus limbatus (2.50 ± 0.78 ppm wet wt), and were the lowest in the fish labeled "Corvina" (0.06 ± 0.05 ppm wet wt). Using the relationships between muscle and liver tissue we were able to estimate THg concentrations (ppm dry wt.) for the livers of these sharks using the known muscle THg concentrations. S. zygaena, which is listed as "vulnerable" by the IUCN – had the highest estimated THg liver concentrations (up to 4.67ppm dry wt.). The elevated Hg levels in the muscle tissues and internal organs (liver) of this species and the other shark sampled have the potential to negatively impact both the health and conservation status of this species as well as having potentially serious health risks to consumers of these products.

146 Assessment of total mercury burden in the American alligator (Alligator mississippiensis) at Merritt Island National Wildlife Refuge (MINWR), Florida

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Mercury is a ubiquitous environmental contaminant that is transported globally via atmospheric circulation. Mercury is toxic in all forms, but the organic form has the ability to bioaccumulate within the tissues of apex predators to concentrations that can be detrimental to these organism's health and fitness in the environment. While many studies focus on seasonal atmospheric deposition trends, studies regarding the seasonal variation of total mercury concentrations in organismal tissues are limited. By comparing seasonal atmospheric and organismal trends, we can determine how fluctuating concentrations influence seasonal behaviors of large predators. Using a long-term sampled population of American alligators (Alligator mississippiensis), we investigate some lingering questions in regard to mercury accumulation and sampling in wild animals. These questions are, if the mercury concentration in an apex predator varies seasonally; if the seasonal variation demonstrated by the atmospheric deposition of mercury is predictive to what is found in apex predators; if both sexes fluctuate similarly with respect of mercury concentration; and, if altered health status is indicative of a higher mercury concentration. To answer these questions, we monitored a constant population of American alligators located at the Merritt Island National Wildlife Refuge in Titusville, Florida, for their mercury concentrations over 7 years. Taking into account the genders, health status, and recaptured individuals, we used precise analytical measurements, spatial analysis, and multiple comparison statistics to identify any significant trends in the present population. We find that there is a statistically significant difference in mercury concentration seasonally, as well as a statistically significant difference in the mercury concentrations of animals with altered health statuses and that total mercury trends that directly correlate with seasonal wet deposition rates for healthy alligators. We present these findings from a model wild population of apex predators to demonstrate the complexity of opportunistic sampling of wild populations as well as lend insight into the effect of seasonal behaviors on samples collected.

147 Effects of trophic position and wetland hydroperiod on mercury accumulation in amphibian larvae

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Bioaccumulation of mercury (Hg) in aquatic food chains is of global concern for wildlife and human health. Previous studies on freshwater fish have demonstrated that Hg accumulation is higher in fish at higher trophic levels in semi-permanent hydroperiod wetlands. The frequency of wetland drying can be positively correlated with Hg accumulation; however, fish only persist in wetlands with infrequent drying. Amphibians, on the other hand, include species adapted to a full range of wetland hydroperiods. Wetland drying and refilling may occur one or more times a year, infrequently every few years, or never in the case of permanent ponds. It is unknown whether Hg accumulation in amphibians is also correlated with hydroperiod. Our objectives were to determine the effects of both wetland hydroperiod and trophic position on Hg levels in larvae of three amphibian species: mole salamanders (Ambystoma talpoideum), marbled salamanders (A. opacum) and southern leopard frogs (Lithobates sphenocephalus). Southern leopard frogs are herbivorous and breed in short to intermediate hydroperiod wetlands. Both salamander species have carnivorous larvae that switch from zooplankton to larval amphibians and aquatic insects during development. Marbled salamanders specialize in wetlands that hold water for no more than 4 to 7 months while mole salamanders require wetlands to hold water for a minimum of 5 months for larvae to complete development. We collected larvae from 25 wetlands on the United States' Department of Energy Savannah River Site near Aiken, S.C. The wetlands represented a range of hydroperiods assessed by a drying score in a previous study. We used a DMA 80 Hg analyzer to measure mercury in 230 freeze-dried, homogenized samples. Overall, both frogs and salamanders in shorter hydroperiod wetlands accumulated more Hg than their counterparts in longer hydroperiod wetlands. Surprisingly, the herbivorous frogs accumulated more Hg than carnivorous salamanders. Within mole salamanders, young of the year larvae accumulated less than paedomorphic larvae that feed at a higher trophic level. From this study it appears that amphibians inhabiting shorter hydroperiod wetlands may be at a higher risk of experiencing the toxic effects of Hg. In addition, herbivorous tadpoles that graze near the wetland sediments may accumulate more Hg than salamander larvae feeding at higher trophic levels, indicating that feeding ecology as well as trophic position may influence Hg uptake.

148 From egg to fledge: Partitioning effects of mercury exposure on reproduction in Great Egrets (Ardea alba)

I.A. Rodriguez, N.E. Vitale, S. Orzechowski, P. Frederick, Univ of Florida / Wildlife Ecology and Conservation

Reproduction in birds is thought to be one of the most sensitive processes affected by mercury (Hg) exposure. Many mechanisms may be involved, including teratogenesis, endocrine disruption, and effects on parental care. Partitioning these effects of Hg has proved elusive in part because the

effects are difficult to study in the same individuals or nests, and in part because effects may be subtle and difficult to assess in complex natural settings. The Everglades (Florida, USA) has a history of mercury contamination that is both temporally and geographically highly variable, making this a good "laboratory" for understanding effects of Hg on a wide-ranging piscivorous bird. In this study, we used nondestructive micro-sampling of egg albumin to determine Hg concentrations before the embryo developed and subsequently sampled the resultant chicks at later stages using blood and feather tissue through fledging. Samples were taken from the 2nd laid egg (0-5 days) and subsequently from blood (≈ 35-45 days) and feathers (≈ 46-60 days) from the same individual in coastal and inland colonies. We sampled albumin from a total of 102 eggs from seven colonies across the Everglades during 2015. No statistical differences were observed in Hg concentrations between coastal and inland colonies in any sampled matrix, and we found no correlation between Hg concentration in albumin and hatching success. However, colony-averaged nest survival (surviving until fledging) was negatively correlated with Hg concentrations from egg (albumin; GLM; p =0.0016), but not to Hg concentration in blood and feathers of chicks. Further, sampled nests with average Hg concentrations in albumin lower than 0.4 mg/kg had nest survival probabilities of 90-95%; in contrast, sampled nests with average Hg concentrations over 0.75 mg/kg had survival probabilities of 42-57%. This information collectively suggests that egg Hg concentrations are affecting nest success, though not through teratogenesis or hatchability. Instead, we suggest that egg Hg is reflective of parental Hg exposure, and that Hg effects on nest success resulted primarily from deficits in parental behavior.

149 Environmental Contaminants Continue to be a Threat to the Endangered Florida Panther

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The Florida panther is a subspecies of puma whose population has been reduced to an isolated remnant population occupying the Everglades and Big Cypress ecosystems of South Florida. Research to investigate the decline of this apex predator identified a number of putative or confirmed morbidity and mortality factors including anthropogenic and intraspecific trauma, inbreeding depression, infectious diseases, and environmental contaminants. Contaminants detected in panthers included mercury (Hg), organochlorines (OC; p,p'-DDE, p,p'-DDT, p,p'-DDD, oxychlordane, dieldrin, heptachlor epoxide, mirex, cis-nonachlor, and trans-nonachlor), polychlorinated biphenyls (PCBs; congeners 18, 66, 118, 153, 105, 138, 187, 128, 180, 170, 195, and 206), and anticoagulant rodenticides (brodifacoum, bromodialone). Concentrations of Hg, OCs, and PCBs were highest in panthers occupying the Everglades ecosystem (ENP), and Hg levels appear to be increasing in some areas. Individual panthers continue to have high Hg burdens, especially in ENP. Although the sample size for ENP is small, concentrations up to 100 ppm have been detected in panthers sampled 2004-2013. Additionally, elevated levels – up to 67 ppm – were detected in southern Big Cypress National Preserve 2008-2013. Restoration of historic water flow and/or shifts in diet may be responsible for this apparent increase. Although white-tailed deer and feral swine are the primary prey species in the northern portion of their range, raccoons comprise an increasingly larger proportion of their diet in the southern portion. This greater dependence on raccoons may result in increased exposure to those contaminants that bioaccumulate through the aquatic food chain. The impacts of these contaminants on panther health and reproduction are unknown. Some panthers with undiagnosed mortalities had elevated Hg, OC, and PCB concentrations at the time of death. More commonly, contaminants likely work additively or synergistically with other stressors to impact panther health and reproduction.

150 Pesticides increase infectious disease by disrupting the microbiome

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Animals are colonized extensively by a community of microbes, which are collectively called the microbiota. Studies suggest that gut bacteria play a role in the development and maintenance of the host immune system, which likely affects their susceptibility to parasites. For example, our previous work demonstrates that an early-life disruption in the microbiota increases parasite susceptibility in frogs. However, we know little about how "real world" environmental stressors, such as chemical contaminants, play a role in these interactions. We tested whether the early-life gut microbiota mediates the effect of contaminants on subsequent parasite susceptibility in Cuban tree frogs (Osteopilus septentrionalis), a species that is common throughout Florida and the Everglades. Tree frog tadpoles were assigned to one of four chemical treatments crossed with either sterile (autoclaved) pond water (to manipulate the microbiota) or non-sterile pond water media (to offer a natural source of microbes). For the chemical treatments, tanks received the fungicide chlorothalonil (1.64 µg/L), the insecticide chlorpyrifos (6.68 µg/L), the insecticide imidacloprid (11.9 µg/L), or acetone solvent control for 7 days. After metamorphosis, frogs were exposed to common parasitic gut nematodes in genus Aplectana. Frogs exposed to sterile pond water had a lower relative abundance and diversity of gut bacteria and, when challenged with worms, they had 5-6 times the prevalence as the pond water controls. All three pesticides had similar effects as sterile pond water, increasing infection prevalence by 5-6 fold relative to the non-polluted, non-sterile pond water controls. Thus, our results suggest that amphibian microbiota mediates the effect of chemical contaminants on subsequent parasite susceptibility. Transcriptomic analyses are underway in an effort to link pesticide-mediated changes to the microbiome to immune gene expression and also changes in immunity to long-term reductions in resistance to infections.

Plastic Debris: From the Continent to the Sea

151 Evaluating Wastewater Effluent as a source of microplastics to the aquatic environment

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In recent years the aquatic environment has been widely affected by microplastics released from various sources and microplastics with a suspected wastewater origin have been detected in marine and freshwater environments in the Europe, US and Australia. However, the amount of microplastics entering the aquatic environment via wastewater effluent discharge remains poorly understood. In this study, we developed a novel sampling device to sample microplastics from wastewater in the size range of ≥500 to 25µm. An efficient sample processing method was also applied to improve the sampling of microplastics in wastewater and to minimize the false detection of microplastics. We quantified and characterized microplastics in the wastewater effluent of three major wastewater treatment plants in Australia with different treatment processes including primary, secondary and advanced (reverse osmosis) treatment. Primary effluent contained on average 1.5 microplastic particles per liter, with lower concentrations of 0.4 and 0.2 particle/L detected in the secondary and advanced treated effluents, respectively. The results also revealed that the dominant particle form were fibers followed by granular particles, which are assumed to originate from textiles and cleansing products. Micro-FTIR confirmed the type of quantified microplastic polymers, which mostly included, PET, and polyethylene This study indicates the importance of wastewater effluent as a source of microplastics to the aquatic environment, particularly given the large daily effluent discharge of the studied wastewater treatment plants.

152 Quantitative and qualitative analysis of microplastics in wastewaters, river and estuarine waters

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Plastic residues constitutes an emerging problem for the aquatic environment including river, estuaries and coastal areas. Their presence is not only an aesthetic problem since they also can imply harmful damages for the coastal areas and the socioeconomic activities linked to these regions. Microplastics (MPLs) are defined as small particles of plastic in the millimetre to sub-millimetre size range (< 5 mm) and high densities (e.g., 100 000 items per m3). In addition, MPLs are suspected to be accumulated and potentially they can enter the food chain. However, their potential occurrence and impact have not been well investigated. One of the reasons for this being the lack of quantitative analaytical methods in the environment. On the other hand, while the study of the impact of plastic debris in marine environment has been considered for years, the impact of smallest fractions as MPLs and nanoplastics, have been poorly studied in the aquatic environment in general, and in freshwater ecosystem in particular. In this context, the main objective of this work was to develop a combined analytical approach for the characterization and quantitative analysis of MPLs. Two polymers were selected among the more frequently used in consumer products: high-density polyethylene (HDPE) and polystyrene (PS). In this platform presentation this combined approach using Infra red spectroscopy (IR), comprehensive two-dimensional gas chromatography coupled with Time-of-Flight mass spectrometry (GCxGC-TOF/MS), matrix-assisted laser desorption/ionization coupled to time-of-flight (MALDI-TOF) and liquid chromatography coupled to high-resolution mass spectrometry (LC-HRMS) using an Orbitrap instrument will be presented and compared in terms of ionization, sensitivity and qualitative information. Among the different techniques, IR spectroscopy was the less sensitive technique, in particular when it was applied to real samples. Regarding to MALDI-TOF, semi-quantitative measurements were achieved but with limits of quantification (LOQ) higher than mg/L. That means that this could be an excellent technique after an improved extraction and pre-concentration steps or for biota samples that usually have higher concentrations of MPLs. LC-HRMS presented so far the best results in terms of quantitative analysis with instrumental limits of quantification (LOQ), between 10 and 100 ppb. Finally, the combined approach was applied to study the occurrence of MPLs in the Delta Ebro River (NE of Spain). The analysis of real water samples from the Ebro Delta detected the presence of PE in influent and effluent WWTP at concentrations of 3.18 and 0.35 mg/Kg, respectively. In addition, the presence of this MPL has been also confirmed in the emissary of St. Carles de la Ràpita as well as in near drainage channels and in three different points of Bahía del Fangar. As regards to the analysis of non-polar semi-volatile plasticizers, the presence of phthalates has been confirmed in all analysed samples The diferent analytical techniques employed will be presented and discussed as well as the results obtained in different real samples from the Ebro river and delta. This work was financially supported by the Spanish Ministry of Economy and Competitiveness, INTEGRA-COAST project (CGL-2014-56530)

153 Sources, Fate and Microbial Interactions of Microplastic Particles in Urban Rivers

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Microplastic particles (< 5 mm) are an environmental contaminant of emerging concern, as recent studies have documented microplastic in marine habitats worldwide. Microplastic sources include fragmentation of larger plastic items, industrial manufacturing pellets, personal care products, domestic cleansers, and synthetic textiles. Rivers have been suggested as a major source of microplastic to global oceans, but there are few measurements of microplastic concentrations or movement in freshwater ecosystems. Our work has documented high microplastic fluxes in urban rivers in the Chicago metropolitan region, ranging from 15,000 to more than 4 million particles per day, and we have identified wastewater treatment plant effluent as a point source of microplastic. Using a longitudinal survey, we demonstrated that microplastic can be transported long distances in a river (> 2 km) and can accumulate to very high concentrations in urban river sediment (10,000 times higher than water column). We also observed dense bacterial colonization of microplastic in urban rivers, and used high-throughput sequencing of 16S rRNA genes to analyze the taxonomic composition of microplastic-attached bacterial communities. Microplastic-attached bacterial communities were distinct in taxonomic composition from communities in associated natural habitats, e.g. water column, seston and benthos. The taxonomic composition of microplastic-attached bacterial communities supports domestic wastewater as a point source of microplastic, as microplastic supported high abundances of bacterial taxa associated with human gastrointestinal infections. Bacterial taxa linked to plastic decomposition were also abundant on microplastic particles, and shifts in microplastic community composition with distance from the WWTP suggest succession towards a ‘stream-like’ bacterial community and away from a ‘WWTP-like’ bacterial community. These results indicate that microplastic represents a novel habitat for bacterial communities and may play a role in dispersal of bacterial taxa within riverine ecosystems. Furthermore, our work suggests that rivers play a major role in the global microplastic life cycle, but the retention, export, and ecological interactions of microplastic in lotic ecosystems are understudied.

154 Microplastic Ingestion By Shape in Several Species of Fish from Lake Ontario

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Microplastics, plastics less than five millimetres in size, come from several sources including industry, consumer products and the breakdown of larger plastics resulting in a number of possible shapes. The anthropic origin of microplastics suggests abundance will increase with increasing proximity to major urban centres. Therefore, Lake Ontario is expected to have relatively high abundance of microplastics. To investigate their prevalence in fish digestive tracts relative to the environment, water samples and fish were collected from three sites in Lake Ontario (Humber Bay, Toronto Harbour and Hamilton Harbour) in 2015. Lab-cultured fish were also exposed to microplastics through diet for a period of 24-48 hours to determine whether ingested microplastics were retained in the digestive tract. Results compare differences in excretion relating to shape. Results indicate that various shapes lead to differences in short-term retention for lab-raised fish. The shapes retained in the highest frequencies differ between species. This suggests that various forms of microplastics may accumulate in the digestive system of several fish species following direct ingestion. Preliminary results from field-collected fish suggest fibres are most prevalent in the digestive tract. These results will be compared to water samples collected from the same locations to determine whether certain shapes are more prevalent in fish digestive tracts relative to the surrounding environment, and which sources may contribute most heavily to the microplastic abundance from these locations. The accumulation and retention of microplastics may cause physical harm and nutritional issues typically associated with macroplastics. Information regarding the most problematic shapes of microplastics and the associated potential sources will aid in informing management decisions and policy development.

155 Manageable sources of microplastics to the "sweetwater seas"

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Microplastic particles (MPPs), plastic particles less than 5 mm in size, present in Great Lakes waters (the sweetwater seas) became a widely publicized issue in 2012, at the same time pressures to remove plastic microbeads from personal care products took hold. However, sampling in the Great Lakes has shown that there are other sources of microplastics entering the lakes from the urban landscape that have commercial and manageable origins. For example, in conducting surface water manta trawls in 2014 in water waters adjacent to the city of Toronto, we found that a portion of the up to 6.7 million particles per square kilometer were comprised of plastic in the forms of polystryene foam, and rigid plastic particles resembling shavings, cuttings, and twistings, and were likely generated by mechanical means. In the current study, we will highlight findings of nearshore Lake Ontario surface water manta net sampling in 2015 that aimed to isolate wastewater and riverine (runoff) inputs within the waters of Humber Bay adjacent to Toronto, as well as river and wastewater treatment plant effluent sampling for inputs to the waterfront. The watersheds adjacent to the Humber Bay area of the Toronto waterfront are known to have a relatively high density of plastics businesses. Findings indicate that waste streams from producers of plastic products are entering wastewater treatment plants and stormwater runoff with subsequent discharge to the lake. This suggests that management strategies that improve best practices that control generation and loss of fine plastic by-product particles within commercial enterprises will further reduce plastics found in the lakes, in addition to anticipated changes from microbeads.

156 Plastics and other anthropogenic debris in freshwater birds from Canada

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Plastics in marine environments are a global environmental issue. Plastic ingestion is associated with a variety of deleterious health effects in marine wildlife, and is a focus of much international research and monitoring. However, little research has focused on ramifications of plastic debris for freshwater organisms, despite marine and freshwater environments often having comparable plastic concentrations. We quantified plastic and other anthropogenic debris in 340 individuals of 17 freshwater bird species collected across Canada. We determined that freshwater birds had similar rates (11.2%) of plastic ingestion to many marine bird species. This work established that plastics and other anthropogenic debris are a genuine concern for management of the health of freshwater ecosystems, and provides a baseline for the prevalence of plastic debris ingestion in freshwater birds in Canada.

157 Assessment of Plastic Ingestion and Persistent Organic Pollutant Concentrations in Sea Turtles from the Pelagic Realm of the Pacific Ocean

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Plastic debris is a growing concern for many marine organisms due to entanglement, ingestion, and exposure to toxic chemicals. We hypothesized that ingestion of plastic debris is a potential source of exposure of persistent organic pollutants (POPs) to threatened pelagic Pacific sea turtles. We necropsied 38 sea turtles [3 leatherback (Dermochelys coriacea), 3 loggerhead (Caretta caretta), 6 green (Chelonia mydas) and 26 olive ridley (Lepidochelys olivacea)] that were incidentally captured in Hawaiian and American Samoan longline fisheries and quantified the amounts and characteristics of plastics in their gastrointestinal tracts. Ingested plastic was found in 87% (n = 33) of the turtles. Mean mass of ingested plastic in all turtles sampled was 9.68 g with a range of 0.0185 g to 64.2 g amongst turtles that ingested plastic. Juvenile green turtles ingested significantly more plastic than other species. Adipose samples from 25 of the turtles (2 loggerhead, 6 green, 17 olive ridley), were analyzed for 83 polychlorinated biphenyls (PCBs), 20 organochlorine pesticides, 32 brominated flame-retardants and hexabromocyclododecane (HBCD). We analyzed differences among species, sex, and correlations with turtle length and capture locations. Total dichlorodiphenyltrichloroethanes (DDTs) were the predominant POP in both loggerhead (mean = 18.3 ng/g wet mass) and olive ridley (15.8 ng/g wet mass) turtles, and the second highest POP class in green turtles (1.80 ng/g wet mass). Total PCBs were the predominant POP in green turtles (2.71 ng/g wet mass), yet had lower concentrations than olive ridley and loggerhead turtles. Green turtles had the highest concentrations of α-HBCD (1.46 ng/g wet mass). Among olive ridley turtles, few sex differences were seen in POP concentrations. Concentrations of several POPs increased with straight carapace length of olive ridleys, suggesting bioaccumulation through age. A geographic gradient was observed with concentrations of several POPs increasing with distance from the equator. Plastic ingestion is extremely common in sea turtles and effects of toxic chemicals could have detrimental effects on their health and survival. Amounts of ingested plastic were unrelated to POP concentrations, suggesting that sea turtle exposure to POPs is predominately through their natural food chain. Additionally, our data provide important baseline POP concentrations for Pacific sea turtles, as this area has not been extensively monitored.

158 Marine microplastics as potential indicators of the Anthropocene

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Marine microplastic pollution is an emerging class of marine pollution that is receiving exponential attention from the scientific community mainly in the current decade. Most concern is related to their accumulation on pelagic habitats, and their environmental impacts to the marine biota their and, more recently, the potential contamination of humans through food resources. Recent surveys showed that plastics are widespread over marine habitats including oceanic trenches and deep sea sediments. Transport and deposition of microplastics over the ocean floor are very slow but efficient processes similar to marine snow, 'faecal express' route and minerals loading; therefore, deep seas are the ultimate repository of microplastics in marine habitats. Because they are highly persistent (i.e., not easily degradable and not completely biodegradable) and clear long-lived on human time-scale, it is expected that they will persist in deep sea sediments for longer periods (i.e., centuries to millennia), being potentially preserved in geological records. Cold temperatures and the lack of UV radiation within the continental shelf – deep ocean gradient magnify plastics preservation. Since plastics have only been produced in large quantities after the II World War, plastic longevity was only inferred through short-time laboratory experiments. As a result, and even more over future, plastics are being incorporated into sediments creating a completely new plastic-rich sediment that mark and identifies human's activities in measurable stratigraphic layers. Regardless of representing one of the most ubiquitous marine pollution forms, microplastics are also a likely indicators of the Anthropocene, a new Epoch to be established within the Geological Time Scale. A number of signs – all anthropogenic in nature, including plastic – are showing that the Anthropocene is indeed stratigraphically distinct from the Holocene. If this premise is confirmed, plastics will be in future 'technofossils' which are trace fossils produced by humans, remaining in the environment for geological time-scales and not for human time-scales as they have been treated until now.

Scientific Advances Supporting Aquatic Life Water Quality Criteria Derivation – Part 2

159 Bringing More "Eco" Into Ecotoxicological Guidance for the Development of Water Quality Guidelines for Metals/Metalloids

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Existing USEPA criteria for most metals and metalloids in freshwater and salt water are built from toxicological approaches that were initially developed before 1985 and refined thereafter. New knowledge of aquatic geochemistry as it affects metal toxicity has been a major focus refining these criteria and is increasingly incorporated into at least some of the criteria in order to add flexibility in dealing with site-specific conditions. A robust "parallel literature" also developed over the last thirty years that illustrates biological and ecological shortcomings in the existing criteria. Unlike geochemistry, nearly all of this literature is formally excluded from consideration by the minimum data requirements for developing criteria. Here it is proposed that a systematic assessment is needed to determine whether a more comprehensive consideration of biological and ecological understanding could help better achieve flexibility as well as more efficiently and effectively protect the environment. Freshwater and saltwater criteria for cadmium and copper will be evaluated as an example to demonstrate some guidelines useful in evaluating the compatibility of the existing criteria with the more comprehensive literature. Questions that will be addressed in the evaluation of the criteria include: Was chronic toxicity, relevant to population processes, adequately addressed? If not what are the implications? Is the justification for species sensitivity robust and is it consistent with field knowledge of such sensitivities? Are the criteria credible when compared with dissolved concentrations known from modern geochemical analyses of contaminated and uncontaminated waters? How do the criteria compare to studies that include dietary exposure? Are the guidelines justifiable based upon field observations that meet criteria for appropriate effects analysis? How do they compare to robust field or integrated laboratory-field observations? A comprehensive evaluation following modified guidelines of this sort could identify criteria least compatible with the full literature and thus most in need of reform. Methodologies and models will be discussed that could be used in a new generation of guidelines that better links biology, ecology and ecotoxicology as well as field observations and laboratory assays.

160 The disconnection between the laboratory and field in understanding the effects of metals on aquatic insects prompts the need to modernize WQC

D.B. Buchwalter, NC State Univ / Dept of Biological Science

The distributions of species that populate natural systems are often quite different in composition from the distributions of species that populate toxicity datasets. Particularly noteworthy is the extent to which aquatic insects are under-represented in toxicity datasets relative to their importance in nature and in ecological monitoring programs. Remarkably, data from only 1 insect species is required for the generation of water quality criteria despite the fact that there are over 7,000 known aquatic insect species in North America alone. When aquatic insect data are examined for trace metal toxicity, it would appear that they are generally insensitive to metals. However, aquatic insects are often some of the first species to disappear from metal-contaminated sites. In fact, typical laboratory results would indicate that insects only respond to dissolved metals at concentrations orders of magnitude larger than those found in the most insect-depleted contaminated sites. Even with mounting evidence highlighting the obvious disconnect between laboratory toxicity tests and field observations regarding metal toxicity to aquatic insects, water quality criteria for metals continues to rely primarily on toxicity values derived from short term dissolved-only exposures. In this talk, I will discuss four key reasons why such tests don't provide relevant data for this important faunal group, focusing upon recent advances in our understanding of bioaccumulation and mechanisms of toxicity.

161 Mesocosms: Old techniques can provide new insight on aquatic life criterion development

T.S. Schmidt, USGS / Colorado Water Science Center; C. Mebane, USGS / Water; L.S. Balistrieri, USGS

Aquatic life critera aim to protect 95% of all species in aquatic ecosystems and those terrestrial organisms dependent on organisms originating in aquatic ecosystems. These ecosystems are dominated both in numbers and diversity by aquatic insects, however, the data used to develop aquatic life criterion rarely include aquatic insects. In part this is because crustacean, amphipods, zooplankton, and some diptera are easily cultured in the lab while most aquatic insects have long lives and complex life histories not suitable for aquaria. Further, beaker-scale toxicological evaluations using standard toxicity testing organisms are criticized for their lack of ecological relevance. We suggest that mesocosms are a technology that can bridge these knowledge and credibility gaps. Here we present data from a series of experiments conducted in the Aquatic Experimental Laboratory, a USGS mesocosm facility located in Fort Collins, Colorado. These data suggest that mesocosm experiments of the effect of aqueous contaminants are replicable, can produce concentration-response curves, and allow for the measure of direct and indirect effects. Thus, ecological, and not just toxicological, endpoints can be evaluated in mesocosm tests. Finally, mesocosm data are extrapolated to multiple field datasets showing that this type of information is reliably transferable to the ecosystems. As a result, mesocosm results can produce beaker-scale type response metrics in ecologically relevant exposure scenarios that are more easily transferable to real ecosystems than standard bioassays. As new technologies and techniques are pursued to advance criterion development into the next century, old techniques might be worth revisiting.

162 Field-based Methods for Developing Water Quality Benchmarks for Specific Conductivity

S. Cormier, USEPA / National Center for Environmental Assessment; L. Zheng, TetraTech

Protection and restoration of conditions that support aquatic life requires chemical and physical benchmarks, standards, or criteria to set loading limits and clean up goals. In 2011, the USEPA published a field-based method for developing an annual average chronic benchmark for specific conductivity (SC). Biological and SC data were used to estimate the extirpation concentrations of a mixture of ions dominated by bicarbonate and sulfate anions. SC levels that lead to 95% extirpation of more than one hundred benthic aquatic invertebrate genera were calculated and used to develop an extirpation concentration distribution to identify the SC that leads to extirpation of 5% of genera. The model was validated with independent data sets and met the characteristics of probable causation. Additional analyses have supported and corroborated the method and it is an EPA-approved method for developing water quality benchmarks. However, the method was silent on parameters usually associated with implementation of benchmarks and water quality criteria. Since 2011, additional methods have been developed to calculate a maximum exposure limit to complement the continuous exposure benchmark. Duration and frequency parameters have been evaluated. Methods have been designed for situations where large data sets of biological and chemical data are not available. These field-based methods are becoming a practical option for estimating benchmarks for common pollutants such as major ions. The views expressed in this abstract are those of the authors and do not necessarily represent the views or policies of the USEPA.

163 Integrating mesocosm experiments and field data to develop water quality criteria for contaminants in aquatic ecosystems

W.H. Clements, C. Kotalik, Colorado State Univ / Fish Wildlife and Conservation Biology

The limitations of single species toxicity tests for predicting effects of contaminants on aquatic ecosystems have been well described in the literature. For certain classes of stressors in which direct toxicological effects are unlikely (e.g., nutrients, suspended sediment, deposition of iron colloids) or in which standard test organisms are highly tolerant (total dissolved solids), field-derived exposure-response relationships have been used to establish standards. Mesocosm experiments provide an ecologically realistic alternative to laboratory toxicity tests while controlling for the confounding variables associated with field-based approaches. In addition to providing mechanistic insights into stressor-response relationships, mesocosm experiments can be coupled with field assessments to address important policy issues. I will present results of two large-scale studies in which mesocosm experiments were integrated with field-based approaches to validate current water quality criteria or benchmarks. In the first example we used field data from spatially extensive surveys of over 300 Colorado streams to establish concentration-response relationships between trace metals and macroinvertebrate community structure. These data were supported by a set of 24 stream mesocosm experiments that allowed us to develop community-level EC20 values for metals. In the second example mesocosm experiments were used to validate a field-based benchmark for conductivity and to quantify the effects of major ions on natural aquatic insect assemblages. This benchmark has been criticized because of the potential influence of confounding variables and the difficulty demonstrating a causal relationship between elevated conductivity and benthic macroinvertebrate responses. Results showed considerable variation among endpoints and salts; however, we observed significant conductivity-response relationships for all major ions tested. These findings demonstrate the strength of integrating descriptive, field-based approaches with mesocosm experiments to establish causal relationships and to identify safe concentrations of contaminants in aquatic ecosystems.

164 Beakers, buckets, and brooks: utility of mesocosm-type studies in aquatic life criteria derivation and validation

C. Mebane, USGS / Water

Since 1985, aquatic life criteria in the USA have been derived using a species-sensitivity distribution (SSD), wherein laboratory toxicity test data derived with a single-species exposed to a single-substance are presumed to adequately represent tolerances of natural communities. While the 1985 aquatic life criteria guidelines allow that microcosm or field data could be invoked to lower a SSD-based calculation, the guidelines did not support the direct use of microcosm or field data to derive criteria. However in practice, only once (1987 with selenium) has a national criterion known been changed based on field data, and recent national criteria documents have ignored community or field data altogether. In contrast, mesocosm or field data are required to validate SSD-based predicted no-effect concentrations (PNECs) under the European Union Water Framework Directive (WFD) and may be used to generate data for the Japan Chemical Substances Control Law. This presentation will illustrate examples where field or experimental ecosystem data have or could be used in conjunction with traditional SSDs, including generating individual species sensitivity data with aquatic insects that would be infeasible to develop from standard laboratory testing.

165 Censoring as a Tool for Optimizing Data Usage in Region-Specific Species Sensitivity Distributions for Hypoxia

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Hypoxia has emerged as a significant environmental issue for the Gulf of Mexico aquatic ecosystem. This study used species sensitivity distributions (SSDs) to evaluate hypoxia sensitivity among marine and tidally-influenced fish and invertebrates of Texas. Representative models require a broad, unbiased sample of species sensitivity data as input. Unfortunately, it is often the case that sensitivity data are only available for a small number of species. The low quantity of data for DO sensitivity among Texas species provided an ideal setting in which to explore methods for increasing the amount of available, standardized data (e.g., acute LC50s). One approach to increasing data quantity is to relax standards for data quality. For example, rapid toxicity testing has been adopted as a way of obtaining approximate sensitivity data on a large number of species. Since smaller sample sizes are used in such toxicity tests, they often result in indeterminate LC50s which can only be expressed as censored values (i.e., less-than, greater-than, or interval values). The current study also relaxes the requirements for data quality as a tradeoff for data quantity; however, an alternate source of censored data is explored — that is, censored data implicit in non-standardized data. Conversion of non-standardized point values into standardized censored values was investigated as a method of maximizing the use of available sensitivity information in the construction of parametric SSDs. Application of this approach increased sample size of the fish dataset from 10 species to 14 species, and the invertebrate dataset from 4 species to 5 species. Inclusion of censored data also shifted HC5s (the concentration protective of 95% of species) upward and downward by varying amounts, although the differences were not significant. The implications of the study are twofold. First, the study identifies a potential store of information that can be used to supplement low quantity datasets used to construct SSDs. Second, it identifies information that may be unintentionally ignored. Authors have discouraged the common practice of discarding censored data. This study demonstrates that even in datasets where censored data are apparently absent, censored data may still be ignored because that information is concealed in non-standardized point values. Furthermore, the results of this study may be valuable in establishing protective DO levels and assessing risk in the Gulf of Mexico.

166 Development of Chemical Thresholds for Situations where Good Ecological Status is unlikely to be achieved

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In the UK, and also likely in some other parts of the European Union, there are challenges in regard to meeting statutory Environmental Quality Standards (EQS) thresholds which are considered to be consistent with good ecological status. As the EQS is a regulatory bright line – you pass, or you fail, it is difficult to gauge the impact status of the waterbody from a chemical perspective. It is also difficult to show that any improvement measures taken in response to EQS failure actually result in a real improvement, because there are no thresholds for lower levels of protection, i.e. an improvement is only registered if the EQS is met. Therefore, thresholds which reflect lower levels of ecological quality could be useful communication tools when looking to demonstrate improvements towards the EQS in situations where meeting it is aspirational. Three metals Fe, Ni and Zn were selected for this study, and three methods were identified to derive "less-than-good" thresholds for lower levels of protection, and for a possible application as targets for impacted environments – i) the use of a higher potentially affected fraction (PAF), from the Species Sensitivity Distribution (SSD) used for EQS derivation, that is hazardous to e.g. 10, 25% of the ecosystem (HC10, HC25), ii) the derivation of an SSD based on a higher effect level (e.g. EC20 or EC50 for chronic effects, rather than EC10 or NOEC), iii) and lastly, the derivation of thresholds directly from ecological data for a relevant metric (e.g. invertebrate abundance and diversity) where related field data are available, using a quantile regression analysis. Because of lack of data, the second method based on a higher effect levels could not reliably provide any sort of conclusion. Nevertheless, less-than-good thresholds as bioavailable HC10 and HC25 metal concentrations were derived from experimental data, and were compared with bioavailable ecological thresholds estimated using benthic macroinvertebrate metrics. Ecological monitoring data of phytobenthos and macrophytes were also used as supporting information. Overall, preliminary results indicated that the use of a less-than-good threshold such as the hazardous concentration to 10% of all species, instead of the currently adopted 5% threshold, would likely be sufficient to ensure an adequate level of protection to ensure the good status of the ecology of the waterbody, i.e. plants, invertebrates, and fish.

Novel Mechanisms of Nanomaterial Toxicity Through Direct Exposure or Indirect Interactions with Environmental Components – Part 1

167 Long term memory (LTM) formation as a non-invasive endpoint to assess copper oxide nanoparticles toxicity in the snail Lymnaea stagnalis

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L. stagnalis has been widely used as a model species in the investigation of linkages between neurobiology, behaviour, learning and memory. The ability of animals to learn and remember enables them to adapt to environmental changes, however, if under stress, their ability to learn and long term memory (LTM) formation may be affected. Because memory is dynamic, stress can positively or negatively alter LTM formation. We hypothesized that stress induced by exposure to nanomaterials, can alter LTM formation in L. stagnalis,and therefore the normal behaviour response to the environment would change. To investigate this hypothesis, we applied a novel assessment method to detect the effect of chronic exposure (30 days) to CuO nanoparticles (NPs) on LTM formation using the freshwater snail, L. stagnalis. Two endpoints were assessed: aerial respiration behaviour and LTM following operant conditioning in the presence of CuO NPs. Results show that aerial breathing behaviour is not affected at the exposure concentrations range (0-250 μg L^{-1} Cu) of the CuO NPs investigated. However, LTM tests were carried out only for the samples collected from controls and 50, 100 and 150 μg L^{-1} Cu exposure vessels. Snails exposed at higher concentrations were not responsive to the operant conditioning necessary to perform the LTM test. Data collected from the test performed on the control organisms showed the ability of the snails to learn and form LTM. However, after 30 days of exposure to CuO NPs at a concentration of 150 μg L^{-1} Cu, snails were not able to learn or form memory. Overall, these findings are in accordance with our previous experiments. Chronic exposure to CuO NPs demonstrated that at concentrations higher than 200 μg L^{-1} Cu, snails show both lethal and sublethal effects in terms of changes in weight and significant decreases in fecundity parameters. In conclusion, we propose this novel approach as a sensitive method to evaluate the toxicity of nanomaterials. Indeed, results obtained are comparable with those obtained with more conventional methods, where costs and numbers of animals used are considerable higher. This research project is funded by the European FP7 project SUN “Sustainable Nanotechnologies”.

168 Enhanced toxicity of environmentally transformed ZnO nanoparticles relative to Zn ions in the epibenthic amphipod Hyalella azteca

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As ZnO nanoparticles (NPs) make their way into the aquatic environment water chemistry will influence their fate, transport, and extent of their dissolution. Although the fate and transport of ZnO NPs is complex, sedimentation is expected in most surface water environments, which will put benthic organisms, including the amphipod Hyalella azteca, at particular risk. In addition, ZnO NPs undergo a complex set of transformations in the environment, including pH dependent changes in speciation and solubility and transformations into phosphates, sulfides and carbonates. The goal of our research is to determine the relative toxicity and uptake of environmentally transformed ZnO NPs to H. azteca and reveal the chemical and physical factors responsible for the differences in toxicity. H. azteca were exposed to ZnSO4, pristine ZnO NPs, and environmental aged ZnO NPs which resulted in three types of particles: 30 nm ZnO-Zn3(PO4)2 core-shell structures, micron scale hopeite-like phase Zn3(PO4)2*4H2O), and ZnS nano-clusters. Exposure conditions included a synthetic freshwater only, increased salinity (3 ppt), and the presence of sediment, with a final exposure where animals were contained within mesh baskets to prevent burrowing in the sediment. Although characterization of the aged particles has shown decreased dissolution of these particles in freshwater, phosphate-aged ZnO NPs had increased toxicity relative to other particles. Saltwater decreased the toxicity of ZnSO4 and all the ZnO NPs, with the greatest reduction in the phosphate-aged particles. In sediments, all treatments are about an order of magnitude less toxic, but when H. azteca are placed in mesh baskets to prevent burrowing, the toxicity is only reduced 3-5 fold relative to the sediment free exposures. Finally, the sulfide aged particles (ZnS nano-clusters) have the greatest reduction in toxicity in the sediment exposures, suggesting binding and possible sequestration of the particles by the sediments. These results confirm previous studies demonstrating that H. azteca is very sensitive to ZnO NPs and the increased toxicity of ZnO NPs relative to ZnSO4 suggest particle-specific effects or enhanced uptake of Zn from the NPs. Because H. azteca is one of the most sensitive organisms to ZnO NPs, there is an urgent need to understand the processes that govern the differential toxicity of these particles. Ongoing studies will address this need by measuring bioaccumulation and biokinetics.

169 Evaluating the novel mechanisms of toxicity of complex nanomaterials across organisms and the impact of organic matter and media components

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Although a variety of nanomaterials have been evaluated for their potential safety, there are questions as to how to use this information to predict the next generation of materials which may be more complex and different in composition. In order to create nanomaterials that are environmentally benign it will be necessary to understand the fundamental molecular interactions that occur between a variety of nanomaterials as well as a variety of organisms in order to minimize negative adverse outcomes. In the Center for Sustainable Nanotechnology (CSN) we are studying several types of next generation materials including complex metal mixture nanomaterials being developed for batteries (including NMC, LiCo and variations of these with differing ratios of metals), NP-polymer combinations, carbon based materials, and ligand-nanoparticle combinations that vary in their charge (AuNP-PAH, AuNP-MPA). For several of these materials we have found evidence of nanomaterial specific responses that are different than the surface ligands on the materials or the ions that may be released during the exposures. These molecular changes include alterations of metabolic pathways, detoxification, growth and reproduction, as well as cell surface functions that may provide insight on the mechanism as well as location of interaction of the nanoparticle with the cells of the organism. We have also compared these results across multiple model organisms in the CSN that represent pelagic as well as sediment dwelling model species. We find significant differences in toxicity across models that are also associated with variation in molecular responses across organisms. This variation may be in part due to differences in the cellular chemistry across species and associated sensitivies. For example vertebrates are more sensitive to manganese and therefore altering concentrations of this metal in battery materials has significant consequence. However, differences seen across model organisms have also been found to be partly due to the different environmental conditions or experimental conditions for each of these species including the presence of organic matter, differences in ions present, or pH which can lead to different states of the nanomaterial present during exposures. The implications for deriving conclusions about the molecular effects of nanomaterials and safety limits across the environment based on different model species will be discussed.

170 Estimating Graphene Oxide Exposure and Biomarker Response in Aquatic Systems

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Anticipated applications and production volumes of graphene family nanomaterials have raised concerns about their potential release into the environment. Graphene oxide (GO) is a promising member of the graphene family due to its aqueous solution-based manufacturing utility and its role as a precursor to the synthesis of other graphene-based nanomaterials. GO structure is similar to that of 2-D graphene sheets, but differs in that its basal plane is populated with hydroxyl and epoxy groups, and its edges with carboxyl groups. Due to its high oxygen content, GO has been shown to be readily dispersible in the water column, leading to concerns about its transport and subsequent toxicity in surface waters. In addition, studies have indicated that GO phototransforms under sunlight irradiation to yield a wide array of products with differing transport and toxicity characteristics. These photoproducts include not only transformed GO nanoparticles but also a solubilized fraction of oxygenated PAHs resulting in the potential for complex exposures that include direct GO exposures as well as indirect exposures to GO photoproducts. In this report, an approach for estimating GO transport, transformation, and biomarker response in surface waters is detailed. GO transport and mass distribution in the water column and in sediments is modeled through application of the Water quality Analysis Simulation Program (WASP8) recently updated to include nanoparticle attachment kinetic parameters. The potential toxic effects of estimated environmental concentrations are then interpreted using two different nanomaterial exposure biomarker protocols utilizing model membrane systems and metabolomics profiling. Model membrane systems include DOPC/DOEPC supported bilayers as well as bilayers derived from fathead minnow (Pimephales promelas) cell membrane extracts. GO interactions with model membrane systems are quantified using quartz crystal microbalance deposition measurements. GO-model membranes interactions are compared with fathead minnow metabolomic profiles to assess the potential ecotoxicity of GO in fresh surface waters.

171 Fish gastrointestinal tracts influence the sorption of organic contaminants to single-walled carbon nanotubes

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Increases in production and commercial use of single-walled carbon nanotubes (SWCNTs) may lead to their release into aquatic environments. While SWCNTs have been shown to be fairly inert, they exhibit high sorption affinity for hydrophobic contaminants that may already be present in aquatic environments. It has been hypothesized that these interactions may reduce oral bioavailability however evidence from our laboratory suggests that sorbed contaminants may still be bioavailable. The synthetic estrogen, ethinyl estradiol (EE2) sorbs to SWCNTs with high affinity, but these mixtures still cause binding and activation of estrogen receptors in vitro, indicating that EE2 maintains bioactivity either through desorption or by preserved interaction with receptors. To determine if these results translate to systemic responses in fish with variable GI tracts, largemouth bass and fathead minnows were orally exposed to mixtures of SWCNTs and EE2. Bass contain a low pH stomach whereas minnows lack a stomach and maintain a relatively neutral pH (7.4) throughout the intestine. Forty eight hours following exposure of fish to EE2-SWCNT by gavage, hepatic vitellogenin mRNA expression was measured as a classic biomarker of estrogenic exposures. Results indicate that EE2 fails to remain sorbed to SWCNTs in fish gastrointestinal systems, irrespective of gastrointestinal physiology, as vitellogenin levels were induced by EE2 in the presenc e of the SWCNTs. As we have obserbed that the SWCNTs do not cross the intestinal epthelium, these resulst imply that the EE2 desorbs from the SWCNTs and is absorbed where it can circulate to the liver and iduce vitellogenin expression. A similar observation was noted in a longer term study where bass were fed SWCNTs-EE2 for 12 weeks. Finally, to begin to elucidate the mechanism of desorption of EE2 from SWCNTs in the GI system we examined the ability of several gut proteins (trypsin, chymotrypsin and pepsin) to displace EE2 from SWCNTs using radiolabeled binding assays. In preliminary studies, trypsin seemed to displace EE2 from the SWCNTs more effectively than other proteins, suggesting that such proteins can imapct EE2-SWCNTs in a protein specific manner. Overall, results of this work futher our understanding of interactions of SWCNTs and contamiants already present in our aquatic environments which is essential for determining the potential organism and ecosystem health risks of these materials.

172 Characterization of the key mechanisms influencing bioavailability of PAHs adsorbed to carbon nanotubes in the aquatic environment

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Concurrent with the high applicability of carbon nanomaterials (CNM) in a variety of fields and the potential use for pollution remediation, there is the inevitable release of CNMs into the environment. Due to CNMs' high adsorption affinity for organic contaminants (OC), there is significant concern that CNMs will act as "contaminant transporters." While adsorption of contaminants from CNMs play a significant role in the ultimate fate of adsorbed compounds, there is little conclusive information characterizing the relationship between adsorption behavior and bioavailability of CNM-adsorbed contaminants. Carbon nanotubes (CNTs) are some of the most commonly used CNMs and though CNTs morphology and adsorbate chemical characteristics are strongly influential of adsorption behavior, it has yet to be established how such factors influence bioavailability of the adsorbed contaminant. The goal of the present research was to characterize the bioavailability of a model class of organic contaminants, polycyclic aromatic hydrocarbons (PAHs), adsorbed to CNTs as a function of adsorption behavior focusing on the influence of CNT morphology and PAH physicochemical characteristics. Adsorption isotherms of a suite of PAHs to CNTs and graphene were established in conjunction with quantifying the bioavailability of the adsorbed PAH to Pimephales promelas (fathead minnow)using bile analysis via fluorescence spectroscopy. Experimental results demonstrated that the presence of CNTs and graphene similarly reduced the bioavailability of adsorbed linear PAHs by ~60%, but only reduced the bioavailability of adsorbed angular PAHs by ~25% and 0%, respectively. This indicates that bioavailability of angular PAHs adsorbed to CNTs is more susceptible to the CNT "surface curvature" effect than linear PAHs. Normalization of adsorption capacity to PAH hydrophobicity and the corresponding bioavailability data further suggest that there may be a size threshold; PAHs beyond a certain size and width will remain more bioavailable than smaller yet less hydrophobic PAHs. While adsorbate hydrophobicity is arguably the most influential characteristic driving adsorption behavior, bioavailability of CNT-adsorbed PAHs, and potentially other planar organic molecules, is strongly influenced by the compounds' molecular configuration and size. This study provides insight into both the potential risk of environmental CNTs and the effectiveness of such materials for pollution remediation.

173 Goldfish (Carassius auratus) immune responses to intravenously injected polymer-coated TiO2 nanoparticles

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Evidence of nanoparticle (NP) effects on immune cell function has been demonstrated using in vitro models in previous studies that report both the over-activation of pro-inflammatory responses, and suppression of others. Our group has also demonstrated modified immune effects for both cell lines and isolated primary goldfish neutrophils when exposed in vitro to polymer-coated metal-oxide NPs. However, the translation of these effects to in vivo models has not yet been explored. Despite a lack of information of in vivo immune effects, it has been shown that when NPs enter circulation in fish, the vast majority is deposited into kidney and spleen tissues, which in fish are the major hematopoietic organ and where many innate immune cells reside. Thus, there is an increased opportunity for neutrophils and macrophages in these organs to interact and be affected by tissue-accumulated NPs. In this study we have sampled kidney and spleen tissues and isolated kidney neutrophils and macrophages at 0, 1, 7, 14 and 28 days from mature goldfish (Carassius auratus) injected with either polymer-coated TiO_2 (1 μg/g) or Cortland's saline (control). Preliminary results show significant and long-term deposition of Ti metal in the kidney and spleen, increased kidney and spleen tissue damage, increased responsiveness of kidney neutrophils and changes in the expression of various pro- and anti-inflammatory related genes in TiO_2-injected fish. We also show a differential response in the capacity of TiO_2-exposed fish to clear a known pathogen during a 28-day infection challenge. Results from our study demonstrate that NP exposure can significantly impact a fish's capacity to appropriately activate numerous immune functions during an infection and this may affect their long-term health and viability.

174 Release of polymer additives and plasticizers from polycarbonate- and epoxy-SWCNT nanocomposites

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Single-walled carbon nanotubes (SWCNTs) have shown considerable promise as filler materials in polymer composites as a result of their excellent mechanical, thermal, and electrical properties. Upon use and subsequent entry into the environment via waste streams, it will be important to ascertain the potential for both carbon nanotube release; as well as, how SWCNTs influence the release of polymer additives and plasticizers from these composites. In this study, we investigated how incorporation of SWCNTs affects the release of plastic additives such as bisphenol A and nonylphenol from polycarbonate and epoxy SWCNT composites under environmentally relevant scenarios. Polymer coupons of varying SWCNT mass loading were subjected to simulated weathering scenarios (e.g. room temperature, elevated temperature, UV light exposure) in both EPA moderately hard water and TCLP extraction media. Aliquots of leachates in the media were taken every 24 hours over the course of five days and were analyzed by LC-MS/MS for polymer additives, while carbon nanotube quantification was carried out by near infrared fluorescence spectroscopy and single particle ICP-MS. Our findings have demonstrated that although the mass loading of SWCNTs in the polymer plays a minimal role in the release of BPA from PC-SWCNT composites, the extraction media significantly impacts the release of BPA from polycarbonate, with releases of BPA leaching exceeding 100ppb over a 48hr time period. Differences in the leaching behavior in different media is likely due to the susceptibility of polycarbonate to hydrolysis at elevated or depressed values of pH. Additionally, single particle ICP-MS has shown minimal release of the SWCNTs from the polymer matrix. Future research plans to incorporate fluorescent CNTs into the polymer matrix in order to quantify SWCNT release by near infrared fluorescence.

Mercury Fate and Biogeochemistry

175 Elemental Mercury: Its uniqueness makes it beautiful yet deadly

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Since ancient times, mercury has fascinated humankind because it has a silver color, it is liquid at room temperature, and it dissolves gold. Due to its fascinating and unique properties, mercury has been used throughout the history for different applications such as barometers, thermometers, electrical switches, fluorescent light bulbs, and ballast for submarines. Mercury is a special element because its chemistry is different compared to other elements. Mercury is considered a global pollutant because mercury in its elemental form has an atmospheric residence time in the range of six months to one year allowing it to travel for long distances, it can be bioaccumulated and biomagnified through the food chain, and in elevated concentrations is highly toxic to humans. Its toxic effects were already known in the 19th century where it was related the occupational exposure of mercury with the Mad Hatter Disease. The first case of severe methylmercury poisoning was discovered in Minamata city, in 1956 where people consumed large amounts of fish and shellfish that were contaminated with methylmercury from the wastewater release of the chemical factory Chisso Corporation to the Minamata bay. The health effects that provoked methylmercury in the people of Minamata were damage to the central nervous system including loss of vision, hearing and speech damage, cerebellar ataxia, and equilibrium disturbance among others. The Minamata disaster and other disasters of mercury poisoning led to the creation of the Minamata Convention Treaty in 2013 whose objective is to protect the human health and the environment from anthropogenic emissions and releases of mercury and mercury compounds. The principal objective of this paper is to evaluate several aspects of Hg^0 behavior and fate in the environment to provide a better understanding of the chemical processes taking place. This review is important because Hg^0 is an important species in the Hg biogeochemical cycling and its behavior in the environment is not well understood.

176 Mercury concentrations, exposure modeling, and isotopic source tracking of mercury in the marine-caged fish farms, South China

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Provinces along the coast of South China have experienced an unprecedented growth in its marine-caged fish industry. We sampled commonly eaten species from two cage-cultured fish farms at the Fujian Province. Total mercury (THg), methylmercury (MHg), and stable mercury (Hg) isotopes were analyzed for fish muscle, sediments, and fish feeds. Reported δ^{202}Hg and Δ^{199}Hg values indicated mass dependent/independent fractionations (MDF/MIF). THg concentrations of the 14 species were all lower than the human health screening value (300 ng/g, wet). There were no statistical differences in Hg concentrations between the cage-cultured and wild caught fish, but the %MHg in cage-cultured fish were lower due to their shorter food chains. Assuming a body weight of 60 kg, the human dietary MHg intake associated with red seabream, Japanese seabass, orange spotted grouper, or eightbar grouper exceeded the USEPA's reference dose (0.1 ug/kg-day). The sediment δ^{202}Hg were low (-1.45‰ to -1.23‰) but the Δ^{199}Hg (-0.04‰ to 0.01‰) were nearly zero. Given its small percentage of MHg (< 3%), it is inorganic Hg that presented such low δ^{202}Hg values, suggesting both atmospheric deposition and microbial activities contributed to the negative MDF signatures. The MIF signatures indicated an industrial Hg source to this region. Hg isotopic values in fish muscle were dominated by diet with pellet-fed fish presenting lower δ^{202}Hg and Δ^{199}Hg values than the viscera-fed fish. The log-MHg concentrations and %MHg positively correlated with δ^{202}Hg values, but not Δ^{199}Hg values. This is because MIF did not occur during metabolic processes and only reflected the signatures of their diets, so fish eating similar food maintained similar Δ^{199}Hg values. Marine-caged fish farms are unique ecosystems, with large input of industrial sources, high DOC, weak photochemical reactions, strong microbial activities in the sediments, high density and simple feeding activities. Though most species had low Hg concentrations, the consumption of several carnivorous fish (e.g., grouper) were of concern. Pellets and viscera feeds provided the major source of Hg to the farmed fish. We suggest using fish feeds, such as shrimp, with low Hg concentrations as an alternative food. With the increasing production of caged-fish in China, our results demonstrate the need for a detailed exposure modeling and risk assessment of Hg through consumption of marine-caged fish.

177 Kinetics of mercury accumulation and its short-term effects towards freshwater biofilms

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Freshwater biofilms are communities of microorganisms, which accumulate and transform Hg, notably through its reduction, methylation and demethylation. The present study aimed to get further mechanistic understanding on Hg and biofilm interactions and focused on the biological parameters that control Hg accumulation in biofilms. To that end, two biofilms of different ages were exposed to ~150 pM Hg (precisely measured) for 24 h in light and temperature controlled conditions using microcosms. Hg uptake kinetics of biofilms were examined with the measurements of non-extractable (obtained after a washing step with cysteine; proxy of intracellular) inorganic and methyl-Hg contents as a function of time. Hg short-term effects were also analyzed at the genomic, physiological and community levels. The old biofilm was characterized by a biomass per cm^2 twice higher than the young biofilm. However, no significance difference was found in their percentage of biotic fraction and chlorophyll a content. The young biofilm was characterized by a higher abundance of the merA gene and a lower abundance of the hgcA gene than the old biofilm. Finally, taxonomic analysis revealed a difference at the microorganism community levels, especially for microalgae. Hg uptake was characterized by rapid kinetics, within minutes, for both biofilms. However, the Hg uptake rate constant of the young biofilm was 10 times higher than that of the old biofilm, probably due to the diffusion limitation of Hg towards microorganisms by the larger thickness of the EPS matrix of the old biofilms. As such, Hg accumulation in the young biofilm plateaued after 2 h of Hg exposure whereas a linear increase of Hg accumulation was observed during the 24 h of exposure in the old biofilms. Algal communities were strongly affected by that short Hg exposure with a decrease in the chlorophyll a content and in the percentage of Chlorophyta present in both biofilms. Effects on bacteria communities were visible at the genomic level with an increase of the merA gene and community level with the increase of Cyanobacteria percentage. The present study evidenced the rapid response of biofilms to Hg exposure in term of accumulation and effects and highlights their possible use as bioindicators of exposure and effects of Hg towards aquatic microorganisms in contaminated freshwaters.

178 Trophic magnification of mercury in aquatic food webs: worldwide patterns and drivers

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The increase in mercury (Hg) concentrations in aquatic organisms with increasing trophic level (measured using d^{15}N), known as the trophic magnification factor (TMF), averages approximately 8 times for methyl Hg, but ranges from 1 to 64 times across diverse food webs. Based on an analysis of 69 food web studies conducted in the last 20 years (before 2013) in marine and freshwater systems, we previously showed that some of this variation is related to latitude, with Arctic food webs having higher TMFs (~10 times) than those from the tropics (~4 times), possibly due to effects of slow growth in animals from the former food webs. Also, areas with high Hg inputs (via atmospheric deposition) tend to have food webs that exhibit lower trophic magnification of this metal, thus lessening risk from exposure in these otherwise contaminated food webs. Yet most of the variation in TMFs across systems remains unexplained. Here we revisit and add new data to this global dataset to further explore these patterns and drivers. We identified an additional 74 studies completed between 2013 and 2016 for potential inclusion in the database. These include studies on under-represented systems such as hydroelectric reservoirs that are known to lead to high Hg in predatory fishes upon dam construction. Given ongoing Hg emissions, deposition and transformations and the Minamata Convention, understanding Hg behavior in food webs at global scales allows for critical links to be made between system characteristics and top predators in food webs that can be used to identify areas at greatest risk from legacy inputs or those that may respond more quickly to global reductions in Hg emissions.

179 Mercury concentrations in northern pike in the Flin Flon, Manitoba area: spatial and temporal responses to smelter closure

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The Flin Flon (Manitoba) copper-zinc smelter was a major metal emitter with an estimated 20 tonnes/yr of mercury released to the atmosphere over the late 1970s to early 1980s. Copper (Cu), cadmium (Cd), mercury (Hg) and zinc (Zn) exceeded Canada's CCME probable effects level guidelines for sediments in several lakes within 8 km of the smelter and did not approach baseline until ca. 70 km distant. However, Hg concentrations in northern pike were lowest in lakes closest to the smelter and highest in two of four lakes 75-85 km distant. Low Hg concentrations in pike close to the smelter were attributed to the inhibition of Hg methylation by metals at toxic concentrations. Metal emissions later declined with improvements in plant design; the smelter closed in 2010. Our study, conducted over 2008-2015, is investigating spatial and temporal trends in Hg concentrations in lake food webs and focusses on northern pike. Despite major improvements in smelter operation, Cu, Cd, Hg, and Zn concentrations in lake sediments close to the smelter are 1.4-2.0 times higher than in the early 1980s and concentrations in distant lakes 1.1-2.0 times higher. These increases are attributable to immense metal reserves on the landscape which continue to enter the lakes through re-emission and runoff pathways. Moreover, Hg concentrations have increased in pike in two lakes close to the smelter, i.e., from 0.09±0.06 µg/g to 0.25±0.16 µg/g for Phantom Lake and 0.12±0.07 µg/g to 0.33±0.19 µg/g for Meridian Lake. These increases suggest that Hg methylation rates at the sediment water interface have increased, possibly because metals have become less toxic with chemical weathering (in the lake and watershed). Percent methyl Hg, a measure of Hg methylation rates, was 2-3% in lake water and similar to lakes (2-6%) in the Thompson area where sediments are not contaminated with metals. Hg concentrations in pike in Lake 1, 74 km to the northeast, have increased slightly (0.18±0.07 µg/g in 1982 versus 0.25±0.14 µg/g) and more so at McClurg Lake (0.63±0.37 µg/g versus 1.00±0.53 µg/g); increases may be related to warming trends and changing water chemistry. Pike condition factor was similar in 1982 (0.68±0.13) and 2008-2015 (0.68±0.08) as was age (5-7 years) with no spatial patterns related to smelter distance; this suggests that high metal concentrations in sediments have had little impact on pike growth rates and longevity.

180 Mercury Accumulation in Laurentian Great Lakes Fish: Are Global Mercury Inputs Affecting the Great Lakes Ecosystem?

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Lake Trout (Salvelinus namaycush) and walleye (Sander vitreus) are collected from two sites within each lake, every other year, and total mercury (HgT) is measured as part of the Great Lakes Fish Monitoring and Surveillance Program (GLFMSP). Previous analyses of Hg concentration trends at these locations were inconsistent, with concentrations at some locations increasing, some decreasing and some not changing. This updated trends analysis indicates that at nearly all sampling locations, HgT concentrations are decreasing with the exceptions of the shallow (nearshore) site in Lake Michigan and the deep (offshore) site in Lake Huron, which showed increasing Hg concentrations. Apparent increasing trends at these two locations are likely the result of older fish present in the size class collected as well as possible localized deposition emanating from nearby industrial centers. These results in conjunction with significant reductions in water column Hg; decreases in Hg(0) concentrations in air over the lakes over the past 7-10 years, and declining trends in Hg wet deposition at most locations near the Great Lakes over the past 10 years suggest that environmental regulations enacted by U.S. and Canada are having positive effects in the Great Lakes ecosystem and that increasing global anthropogenic emissions are not overwhelming regional controls and significantly impacting mercury concentrations in the Great Lakes. Analysis of stable Hg isotope results, which is a new tool in Hg research that can help discern differing Hg sources, pathways and processes will also be presented.

181 Gaseous mercury flux from salt marshes is mediated by solar radiation, temperature, and moisture

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Salt marshes are ecologically sensitive ecosystems where mercury methylation and biomagnification can occur. Understanding the mechanisms controlling Gaseous Mercury Flux (GMF) from salt marshes is important to predict the retention of Hg in coastal wetlands and project the impact of environmental change on the global Hg cycle. We monitored GMF from a remote salt marsh over 9 days which included three cloudless days and a 4 mm rainfall event. We observed a cyclical diel relationship between GMF and solar radiation. When measurements at the same irradiance intensity are considered, GMF was greater in the evening when the sediment was warm than in the morning when the sediment was cool. This is evidence to suggest that both solar radiation and sediment temperature directly influence the rate of Hg(II) photoreduction in salt marshes. GMF could be predicted from solar radiation and sediment temperature in datasets collected during cloudless days ($R^2 = 0.99$), and before ($R^2 = 0.97$) and after ($R^2 = 0.95$) the rainfall event but could not account for the lower GMF after the rainfall event (in contrast to greater GMF from soils after rainfall events) that we propose is due to increased photooxidation or decreased photoreduction in chloride-dominated aqueous solutions.

182 Methylmercury entry and accumulation in the food web of a semi-arid river-reservoir system

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Water management can be a major driver of mercury (Hg) bioaccumulation and risk, particularly in semi-arid western environments where available water resources are managed for often competing environmental and societal uses. Reservoirs in particular have been associated with elevated mercury bioaccumulation because of the effects of reservoir management on the biogeochemical factors regulating mercury cycling and bioaccumulation. The Snake River ecosystem is among the most heavily managed rivers in the US. It receives substantial agricultural drainwater and contains a series of reservoirs along its 1700 km extent. As part of a multidisciplinary effort to understand the drivers of mercury cycling and biomagnification along the Snake River we examined temporal and spatial patterns of methylmercury concentrations at the base of the food web (zooplankton and benthic invertebrates), as well as bioaccumulation through the fish community. Preliminary results indicate that fish Hg concentrations were substantially elevated in the reservoirs in comparison to upstream and downstream river segments. In the reservoirs, methylmercury concentrations in zooplankton exceeded those of benthic invertebrates but there was strong temporal variability in zooplankton methylmercury concentrations. Zooplankton concentrations were lowest in early spring (March/April) and midsummer (July/August), and highest in late spring (May/June) and late summer/early fall (September/October). Although relative changes over time in zooplankton methylmercury concentrations were consistent throughout the study area, there was substantial site-to-site variation during any given sampling event, and concentrations varied by up to 3-fold across sites. Sites that were strongly stratified with substantial hypolimnetic development had higher zooplankton methylmercury concentrations than unstratified sites, thus we evaluated zooplankton methylmercury concentrations within discrete depth strata. Zooplankton methylmercury concentrations were substantially higher at depths that were near the thermocline than those from the upper epilimnion. However, diel sampling suggested that vertical zooplankton migration may be transporting methylmercury from lower depths to the upper epilimnion, increasing potential exposure to fish and other higher order consumers. Our preliminary results suggest that seasonally dynamic processes influence methylmercury cycling through the Snake River food web.

Soil Contaminants: Fate, Bioavailability, Environmental Toxicology and Risk Assessment – Part 2

183 The long term effects of trace metals on soil microbial functions: Biological and chemical implications

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Soil microbial functions are vital for maintaining soil health. There is a lot of evidence showing that soil microbial functions are affected by trace metal contamination in the short term, but that over time they are restored. The long term effect of trace metals on soil microbial functions are however poorly understood. Much of the available data on adaptation is derived from studies focused on European agricultural soils. This study used North American soils across a range of land-uses to identify differences in the adaptation rate and sensitivity. This study aimed at evaluating the methods used to assess the hazards from trace metals using spiked soils. First soils (n=18) were spiked with zinc (Zn) and copper (Cu),8 doses were applied. Then dose-response curves were determined for soil nitrification and enzyme activity (dehydrogenase; arylsulphatase and acid phosphatase). This showed land-use was a factor ($P < 0.01$) effecting the toxicity of Zn & Cu. Boreal forest soil nitrification rates were the most sensitive end-point to Zn (EC50 = 202 ± 44 mg kg^{-1}) and Cu (EC50 = 197 ± 23 mg kg^{-1}). Across all land-uses and soil types a similar pattern of sensitivity was seen: Potential nitrification>dehydrogenase> arylsulfatase> acid phosphatase. Soils were then monitored to identify adaptation of these microbial functions over 180 days. All soils and measurement end-points showed adaptation to both Zn & Cu. The relative sensitivity of each end-point remained consistent after adaptation had occurred. Secondly this study tested whether adaptation to a server stress (trace metals) affects resistance or resilience to a subsequent mild stress. The resistance, resilience and relative soil stability index (RSSI) of soils were determined for heat (60°C for 24 hrs) and moisture stress, applied as short term secondary stresses. This showed that soils which have adapted to Zn & Cu are not less resilient to additional stresses after adaption, than before recovery. This experiment suggests that metal concentration is not a good indicator of soil microbial health but activity is. Additional the effects of primary and secondary stresses on ecosystem functioning are complex and dependent on the effect of the two stresses. Finally this study assessed metal speciation of the artificially contaminated soils. Metal speciation was determined using k-Edge X-ray absorption spectroscopy. Zinc k-edge EXAFS were measured at the HXMA beamline (06-ID1) of the Canadian Light Source Inc.

184 Bioaccessibility and acute toxicity of bismuth to the earthworm Eisenia andrei

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Bismuth (Bi) is increasingly used to replace lead in several industrial applications including the production of alloys and munitions formulations. However, little information is available on the environmental fate and ecological effects of Bi. The present study describes the 14 days acute toxicity of Bi, added as Bi citrate to a natural sandy soil, to the adult earthworm Eisenia andrei. The total measured Bi concentrations were 298, 399.5, 431, and 469.5 mg Bi/kg dry soil. Data indicates that Bi was toxic to E. andrei, as determined by LC_{50} and LOEC, i.e., 416 and 399.5 mg Bi/kg dry soil, respectively. In the presence of E. andrei for 14 d, the bioaccessible fraction of Bi in soil, as determined in KNO_3 aqueous soil extracts, increased by a factor ranging from 1.6 to 30.0 compared to those measured at t=0. Moreover, the increase in soil Bi bioaccessibility was accompanied by a rise in pH and with a increase in mortality of E. andrei. For example, when Bi bioaccessibility increased from 0.262 to 7.516 mg Bi/kg dry soil, the mortality rate increased from 0 to 79 %. We assume that there were at least two routes by which E. andrei enhanced Bi bioaccessibility; one route was guided by the mobility and the biochemical (mucus and bacteria) interactions of E. andrei with soil constituents, and the other route was marked by the death of earthworms and the release of the accumulated Bi from the carcass.

185 Heavy Metals Contamination at the Agbogbloshie Electronic Waste (E-waste) Site in Ghana – Risks to Ecological and Human Health

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There is worldwide increase in the discarding of electrical and electronic equipment (e-waste). Studies at e-waste recycling sites, which are mainly situated in low- and middle-income countries, have started to document ecosystem contamination by heavy metals and organic compounds. Despite concerns, the extent and variability of contamination within a site as well as potential environmental and human health risks is still limited. The objective of this study was to evaluate surface soil at the Agbogbloshie e-waste site (Africa's largest e-waste recycling site) in Accra, Ghana for 19 metals. Within Agbogbloshie, we sampled sites in which e-waste was sorted (n=7), dismantled (n=6), and burned (n=9), as well as in nearby community areas (n=2) and surrounding points (n=26). The soil metal data were analyzed by calculating the enrichment factor (EF) and geoaccumulation index (Igeo) for assessing metal contamination, and the Hazard Quotient (HQ) as a measure of health risk. EF values between 0.5 and 1.5 indicates that the metal is from natural processes or crustal material, while higher values indicate that the metal is from external sources. In average high EF (132-3) was found in all the sampling sites for Cu > Ag > Pb > Cd > Zn > As. The highest EF factors were found in the dismantling areas (EF 362-4) followed by the areas associated with e-waste sorting (EF 318-4) and burning (EF 160-16). For the community sites EF values ranged between 2 and 3. While EF indicates the source of enrichment, Igeo indicates the level of soil contamination with 0 being uncontaminated and > 6 extremely contaminated. The samples from the dismantling and sorting sites had the highest levels of contamination for Cu > Ag > Pb > Cd > Zn As > Se (Igeo 8-2), followed by the burning sites (Igeo 3-7). Soils in the community site were moderated contaminated (Igeo ≤1) by Zn > Cu > Pb > Ag. Concentrations of metals also reach the sample sites located outside the recycling area with EF > 10 and Igeo > 3 for Cu > Ag > Pb > Cd > Zn. The HQ values only exceed the unity for Pb and Cu in the sorting and dismantling site. The Hazard Index (HI =) presented values above 1 for the 3 sites inside the e-waste. Based on EF, Igeo, and HQ, Cu and Pb are the chemicals of greatest concern for environmental and human health, principally in the recycling areas.

186 Does Lead toxicity to the soil microbial community decrease ecosystem service quality?

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The soil biotic community provides several benefits to people. These benefits are termed Ecosystem Services and include food or water provision, and nutrient cycling. The soil microbial community forms an integral part of these processes. Lead exposure, among other metals, inhibits the activity and function of microbial communities which could potentially reduce their ecosystem service performance. Preliminary experiments have shown that lead inhibits the activity of β-D glucosidase and ammonia monooxyenase enzymes. Further studies are being undertaken to understand how this enzyme inhibition affects the xenobiotic degradation potential, climate regulation ability and the organic matter decomposition rate of metal (Pb) stressed microbial communities. To achieve this, 100 soils have been collected from Alberta, Manitoba, Saskatchewan and Yukon. Each of these soils will be spiked with a dose of lead nanoparticles that inhibits at least 50% of β-D glucosidase activity (EC_{50}). Elymus lanceolatus will be grown in the soils for two weeks per Environment Canada's protocol to determine the metal's secondary effects on forage. Forage quality will be determined by a measure of crude protein and biomass. The effects of lead on the climate regulatory role of the microbial communities will be determined by measuring CO_2, CH_4, N_2O using an FTIR. The lead contaminated soils will be re-spiked with glyphosate and the degradation rate will be determined by a measure of mineralized $^{13}CO_2$. The relationship between quantified sub lethal effects of lead on microbial activity and function, and ecosystem service quality will be established at the end of the project.

187 Revisiting the Avian Eco-SSL for Lead: Recommendations for Revision

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The avian Eco-SSL for Pb is very low and within background for most of the US. Site-specific risk-based remedial goals for Pb are frequently many times greater. The extremely low nature of avian Pb Eco-SSL limits its utility for screening purposes. Sensitivity analysis indicates that TRVs play an inordinate role in defining Eco-SSLs. The avian TRV used for the Pb Eco-SSL is based on reduced egg production in Japanese quail. Additional analyses shows egg production in Japanese quail to be very sensitive to Pb, displaying effects at doses lower than other species. The Japanese quail TRV may bias the avian Eco-SSL low. Avian toxicity data supporting the Eco-SSL were re-evaluated. Only studies that reported both NOAELs and LOAELs for reproduction, growth, and/or survival were considered. Dose-response data and supporting information were extracted from each study as concentrations and as dose. Response data were recorded as reported by authors and control normalized. Dose-response relationships were developed for dietary concentrations and doses for reproductive, growth, and mortality effect endpoints. Effect levels (10%, 20%, and 50%) were extracted from dose-response analyses. Data were extracted from 8 studies representing 3 Pb forms and 4 species. Data were sufficient to fit curves for egg production (quail, chicken), growth (quail, chicken), and survival (kestrel). Reproduction was most sensitive and survival least with growth intermediate. Japanese quail were more sensitive than chickens, displaying greater variability. In addition, quail data were not always consistent. Limited data for turtle doves and kestrel suggests lower sensitivity. Chicken data appear less variable. Concentrations toxic to chickens were consistent with levels causing physiological effects in other birds. EC_{10} and EC_{20} thresholds for chickens were 3.6 and 7.8 mg/kg/d. Current NOAEL based on quail (1.63 mg/kg/d) approximates quail ED_{20} (1.2 mg/kg/d). Pb Eco-SSL was recalculated based on ED_{10} and ED_{20} and with and without a bioavailability adjustment. Revised avian Pb Eco-SSLs ranged from 29 to 140 mg/kg. Recommendations: 1) Mine the data in the Eco-SSL reports; 2) Extract dose-response data/investigate relationships for other analytes; 3) Re-calculate Eco-SSLs based on dose-response analyses.

188 Effects of chemicals on soil organisms in the field: available methods and ecological background

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According to European regulations, the effects of chemicals on soil organisms are studied almost exclusively in the laboratory, i.e. in single-species-tests under well-standardized but highly artificial conditions. In case a risk cannot be excluded, higher-tier tests, i.e. field studies or monitoring approaches could be required but in reality only the earthworm field test (ISO 1999) was regularly performed as part of the risk assessment of pesticides (PPP). According to recent discussions within the European Food and Safety Authority (EFSA) and the European Medicines Agency (EMA), the number of more complex field studies may increase in the foreseeable future, for two reasons. In the case of the PPPs, new regulation (EI 1107/2009) requires the protection of biodiversity – a task which has been interpreted by EFSA as the protection of ecosystem services provided by organism communities. In the case of VMP appropriate risk mitigation measures (RMM) have to be identified for those parasiticides which are classified as being persistent (P), bioaccumulative (B) or toxic (T) in the environment. One possibility in such cases would be a post-authorization monitoring. It is difficult to assess the outcome of monitoring approaches, i.e. to decide whether the protection goals are fulfilled or not (in this case whether the functions and services provided by soil organism are provided or not). Data gained at monitoring sites (e.g. the species composition of an earthworm community) has to be compared with reference data (or Normal Operating Range (NOR)). Such NORs have to be defined beforehand at ecologically comparable but non-impacted sites. In other words, the composition and functions of (temperate) soil organism communities or at least their most relevant groups have to be known. In this presentation soil organism communities of temperate regions will briefly be described, including criteria how to identify the most relevant organism groups and their functions (most prominently: organic matter decomposition). Potentially useful methods and endpoints for measuring them are presented, including sampling and identification techniques. Special emphasis will be given on the validation and standardization of these complex methods. Finally, different ways of presenting and evaluating the outcome of monitoring activities will be presented, using examples from European sites.

189 Soil invertebrate avoidance behavoir from a hydraulic lubricating oil

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Avoidance tests are a rapid toxicity test type in soil toxicology to assess the influence of contaminants on organisms behaviors. Behavioral studies are currently lacking in ecotoxicology risk assessment and can easily be incorporated into the common risk assessment tool, the species sensitivity distribution curve. Soil invertebtrate with chemosensing abilities avoid nonvolatile, volatile and semi volatile contaminants. Semi volatile contaminants like petroleum hydrocarbons interfere with chemosensing and alter the distribution and behavior of soil invertebrates populations. Development of guidelines considering ecologically relevant endpoints are important to maintaining ecological functioning of soil invertebrate communities following contamination events. The EC50 values for avoidance tests for numerous soil invertebrates were compared to the EC50 value for reproduction. Soil remediation guideline derived from a SSD was determined with and without inclusion of avoidance tests to assess how these tests influence the resulting guidelines.

190 Effect of select perfluorinated compounds on hatching success of, and accumulation in, the house cricket (Acheta domesticus)

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Perfluorinated compounds (PFCs) are persistent organic pollutants. PFCs are used in a variety of industries including aerospace, automotive, manufacturing, electronics, and textiles. Multiple research studies have provided information regarding concentrations of PFCs in human blood, various mammals, and aquatic life. There is a noted lack of studies regarding the potential terrestrial impacts of PFC contamination. In order to fill this gap, the effect of perfluoro-1-butanesulfonate (PFBS), perfluro-n-octanoic acid (PFOA), perfluoro-1-hexansulfonate (PFHxS), perfluoro-n-heptanoic acid (PFHpA), and perfluoro-n-nonanoic acid (PFNA) on adult accumulation and egg hatching success of the house cricket (Acheta domesticus) was studied. Fertilized cricket eggs (n=20/replicate) were exposed to PFCs in lab-grade sand to evaluate a scenario in which PFCs were readily bioavailable. Each treatment concentration (n = 5; from 0 to 70 µg/g) included 4 replicates except for the high dose. Results suggest that these selected PFCs are toxic to cricket eggs at concentrations ≥ 42 µg/g; these toxic effects are manifested as a decrease in hatching success. Select cohorts that successfully hatched were reared and observed to determine gender ratio and PFC body burdens. Initial results show that PFCs are taken up into the egg and subsequently found in reproductive age adults depending on the chemical and the exposure concentration. Initially, there appears to be some differences in the accumulation of PFHxS and PFOA in adults compared to PFBS. Understanding PFC body burdens in adult crickets following egg exposure should aid in predicting the transport of PFCs through terrestrial food webs.

Frequent Fliers: Effects of the Deepwater Horizon Oil Spill to Birds

191 Deepwater Horizon Oil Spill NRDA Avian Toxicity Studies – Introduction

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On April 20, 2010, the Deepwater Horizon (DWH) drilling rig exploded, caught fire, and eventually sank in the Gulf of Mexico. The explosion and fire killed 11 workers and injured 17 others. For 87 days, oil and gas flowed uncontrolled from BP's Macondo well, creating the largest offshore oil spill in U.S. history. Under the Oil Pollution Act of 1990, Federal, State, and Tribal Natural Resource Trustees are authorized to conduct a Natural Resource Damage Assessment (NRDA) to assess injuries to natural resources and recover damages to restore, replace, rehabilitate, or acquire the equivalent of those resources injured by an oil spill. As part of the DWH NRDA, the Trustees relied on two primary approaches to assess injuries to migratory birds. The first, the Shoreline Deposition Model, used data on the deposition of impaired and dead birds on shorelines along with other data to estimate mortality across the affected area. The second, the Live Oiled Bird Model (LOBM), estimated injury to birds that were oiled, but died later in the spill timeframe. At the most basic level, the LOBM relied on three primary inputs: 1) the numbers of birds in affected areas, 2) the incidence and degree to which birds were oiled, and 3) the fate of oiled birds (i.e., the likelihood a bird would die or suffer other adverse effects). Studies were undertaken to generate LOBM inputs for major bird groups affected by the spill. Abundance and oiling rates were determined by aerial and/or surface-based surveys. Key to understanding oiled bird fate were data on DWH oil-related health effects in field-collected birds. Specifically, the occurrence of hemolytic anemia in birds from oiled areas was clearly important to understanding bird fate. The Trustees therefore undertook four controlled dosing study approaches to help characterize the breadth of toxicological effects occurring concurrently with documented levels of hemolytic anemia to improve oiled bird fate predictions: 1) Oral dose-response studies; 2) External oiling dose-response studies; 3) Metabolic, thermoregulatory, and flight performance effects studies; and 4) Field-based flight effects studies. The following series of presentations describe, in part, the findings of those studies.

192 Overview of Avian Toxicity Studies for the Deepwater Horizon Natural Resource Damage Assessment

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In the wake of the Deepwater Horizon oil spill, federal and state agencies conducted a Natural Resource Damage Assessment (NRDA) under the authority of the Oil Pollution Act of 1990. A component of the NRDA included a series of avian toxicity studies. The goal of these studies was to evaluate the impacts of low to moderate oil exposure and potentially repeated oil exposure that did not result in short-term mortality but might result in physiological impacts that ultimately could affect avian survival and health. Four groups of avian toxicity studies were proposed by experts convened by the Department of the Interior. These studies included oral dosing studies, an external dosing study, metabolic effects studies and field-based flight studies. The objectives of the oral dosing studies were to develop dose-response relationships in multiple species that could be used to predict the toxicity of oil ingested by birds following the spill, investigate endpoints that might be linked directly or indirectly to the occurrence of hemolytic anemia (a finding in oil-exposed wild birds) to provide relevance to field studies and to determine oral doses for the metabolic studies. The objective of the external dosing study was to develop quantitative relationships between external oiling of birds (percent cover, amount, location) and internal dose. The objectives of the metabolic effects studies were to determine the adverse effects of oral dosing on energetics and metabolism including flight performance, thermoregulation, food/energy assimilation and body composition and to measure the effects of feather oiling on flight performance. The objective of the field-based flight studies was to determine if oral and external doses of oil affected performance in homing pigeons trained for long-distance free flights. Results from these studies and their significance in terms of physiological impacts that ultimately could affect avian survival and health are provided in subsequent presentations.

193 Evaluation of blood parameters as a measure of physiological injury to oiled birds from the Deepwater Horizon oil spill

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The Deepwater Horizon oil spill released an unprecedented volume of crude oil into the Gulf of Mexico. While avian mortality associated with this and other oil spills has been documented, the sublethal physiological effects of oil exposure on birds with moderate and light oiling is not yet clearly understood. We found that American oystercatchers, black skimmers, brown pelicans, and great egrets captured during the spill with small amounts of visible oil present on their feathers suffered from oxidative injury to erythrocytes, had decreased numbers of circulating erythrocytes, and showed evidence of a regenerative hematological response in the form of increased reticulocytes (i.e., young red blood cells). We found Heinz bodies present almost exclusively in birds from sites impacted with oil during the 2010 spill – a change pathognomonic for oxidative injury. Further, we found that packed cell volume values were 4% to 19% lower in birds with visible oil than birds from reference sites and that there were 27% to 40% more reticulocytes present in birds with visible oil than birds from reference sites. Together these findings provide evidence that modest oil exposure, based on visible oiling of feathers, can cause hemolytic anemia. Furthermore, we found that birds with no visible oiling captured from the area potentially impacted by the spill also had Heinz body formation, increased numbers of reticulocytes, and reduced packed cell volumes when compared to birds from reference sites. This observation suggests that even birds without visible oil suffered physiological injury from the spill, with ingestion of oil or oil-related contaminants during foraging or preening as a likely additional route of exposure. Anemia causes a decrease in oxygen availability to tissues, muscle fatigue, lethargy, decreased energy availability, adverse reproductive impacts, and may have implications for survival and fitness. In addition to the hematological effects we found in birds from areas of potential impact, our results also indicate that visible oiling is a better measure of potential physiological impact than circulating polycyclic aromatic hydrocarbon concentrations in the blood, suggesting that visible oil evaluation should be a key component of damage assessment efforts.

194 Effects of Sub-Acute External Exposure to Deepwater Horizon Oil in the Double-crested Cormorant

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The Deepwater Horizon (DWH) oil spill presented new challenges in how deep-water drilling affects the environment. Key differences affecting wildlife included that oil dispersed from 70 km offshore and the active spill continued for several months, resulting in potential for repeated sub-acute exposure of wildlife to oil. Previous DWH fieldwork indicated subacute exposure to oil may result in hemolytic anemia as well as the more typical suite of impacts of oil exposure associated with thermoregulation, body condition, and organ injury. We evaluated physiological effects in Double-crested Cormorants (Phalacrocorax auritus) externally exposed (n=13) repeatedly to sub-acute levels (13 g/exposure) of artificially weathered DWH petroleum crude oil compared to control birds (n=12). Hematologic values, thermography, body weight, and internal body temperature data were collected across multiple time points and compared using linear mixed effects regression models with a repeated measures structure. Organ weights and tissue were collected at necropsy. Sub-acute external exposure to oil resulted in hemolytic anemia associated with development of Heinz bodies and reduced packed cell volume. Over the study period, we found that WBC, monocyte, and lymphocyte counts were significantly greater ($p < 0.05$) in oiled birds. At necropsy, we documented gross abnormalities in the hearts of oiled birds. Absolute and relative liver, kidney, and gastrointestinal track weight was greater ($p < 0.05$) in treated cormorants, with evidence of associated oxidative damage to both liver and kidney tissues. Internal body temperatures were stable over the course of the study period for both control and treated cormorants; however, infra-red thermal images of oiled cormorants indicated significantly ($p < 0.05$) greater surface temperatures that resulted in greater heat loss in treated cormorants. We presume increased energetic demand to maintain internal body temperature was compensated through observed increased food consumption in oiled birds. Both oiled and unoiled groups maintained or gained weight during the study period. In this study, repeated exposure to sub-acute levels of DWH oil resulted in multiple negative physiological impacts to cormorants.

195 Using a bioenergetics model to predict increased thermoregulatory costs from oil exposure in Double-crested Cormorants

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The adverse effects of oil spills on waterbirds is well documented. One important effect is the reduction of body insulation as feather structure is altered by exposure to oil. At the extreme, this leads to death from hypothermia; however, there are likely sub-lethal effects such as increased thermoregulatory costs that can draw resources away from growth, survival, and reproduction. Here, we present a novel use of a bioenergetics model to predict and quantify such chronic, sub-lethal effects. Niche Mapper™ is a heat-balance model that uses information about an animal's morphology and microenvironment to calculate metabolic rates necessary for the model animal to maintain its body temperature while accounting for heat exchange with the environment. We validated Niche Mapper's performance against the physiological response of captive Double-crested cormorants (Phalacrocorax auritus) externally exposed (n=13) repeatedly to sub-lethal levels (13 g/exposure) of artificially weathered Deepwater Horizon oil compared to control birds (n=12) over a 21-day period. We measured physiological response using thermography, body weight, food consumption, and internal body temperature data. Niche Mapper's cormorant model accurately predicted surface temperatures for unoiled birds and birds in which plumage properties were parameterized for effects of repeated exposure to oil. We then simulated food intake in control and oiled cormorants to compare predicted differences in food requirements to observed food consumption. Actual food consumption after exposure to 3-4 doses of oil was 13.2% more than unoiled birds, compared to the model predicted 11.7%. Birds exposed to 5-7 doses of oil consumed 23.6% more food than unoiled birds compared to a predicted 15.3% increase. Multiple factors may account for lower predicted food consumption by Niche Mapper, particularly at higher exposure levels. Regardless of causation, modeled estimates of increased energetic demand due to oil exposure may be conservative with respect to actual demand. We are currently mapping and will discuss predicted thermoregulatory costs from multiple levels of oil exposure for cormorants across their range in the eastern United States and how annual life history stages (wintering, migration and breeding) may be affected by these increased energetic demands. The ability to predict such sub-lethal effects helps to provide a more complete understanding of the consequences of oil spills for wildlife.

196 Cardiovascular effects of Oral and Dermal Oil Toxicity Testing in Double-Crested Cormorant (Phalacrocorax auritus)

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At necropsy, double-crested cormorants (DCCO) orally dosed with artificially weathered Deepwater Horizon oil showed signs of cardiopathy and extended clotting time. Methods for assessment of pathophysiologic endpoints and functional imaging of DCCO hearts were developed. In this study mixed-sex DCCOs had oil (test, n=13) or water (control, n=12) applied to the breast and back feathers every 3 days, covering approximating 20% of the body surface. Techniques modified for avian species included echocardiography, activated clotting time, and troponin I analyses, in addition to previously developed light and electron microscopic evaluation of anemia. Activated clotting time of externally oiled cormorants was significantly increased over control cormorants, indicating coagulopathy. Opportunistic fecal cytology comparing treated versus control birds documented hematochezia (external blood loss through the feces) in treated birds only. Echocardiography revealed a significantly increased left ventricular chamber size and therefore decreased myocardial contractility both between treated and control birds and treated birds before and after oil exposure. Echocardiography also revealed significant cardiac arrhythmia, including probable ventricular tachycardia, in treated birds only, which could result in mortality. Troponin analyses revealed a significantly increased plasma concentration in treated birds compared to their pretreatment values. In summary, myocardial dysfunction, cardiac arrhythmia, and probable coagulopathy were documented in oil-treated birds only.

198 Oil and the Long Distance Migrant: Flight Patterns, Behavior, and Body Mass is Altered Following Exposure to Oil from the Deepwater Horizon Oil Spill

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The three month long Deepwater Horizon oil spill left thousands of birds dead; however, the fate of thousands of other birds that were affected but did not die as a direct result is unknown. This study used the homing pigeon as a surrogate species for migratory birds to investigate the effects of a single external oiling event on their flight performance. Homing pigeons were equipped with GPS data loggers and released 100 miles from their home loft for a series of baseline and experimental flights. After completion of the baseline flights, MC 252 crude oil was applied to the wing and tail feathers of the birds in the treated group (approximately 20% of their surface area). Repeated experimental flights followed the single application of oil and flight performance parameters were recorded. Data from the GPS loggers showed that lightly-oiled homing pigeons flying repeated 100 mile flights resulted in altered flight paths, increased flight duration and increased flight distance. The oiled birds were also unable to regain body mass between flights to the same extent as the control birds. These data suggest that in addition to taking more time to complete flights, the inability of oiled birds to recover their mass between flights would require more time spent at stopover sites, further slowing migration and causing delayed arrival to destination sites. Delays during migration have been shown to cause reductions in reproductive success and survival. This work was funded in part by the U.S. Department of Interior's Natural Resource Damage Assessment for the Deepwater Horizon/Mississippi Canyon 252 Oil Spill and the Nevada Agricultural Experiment Station.

Arctic Spill Science – Fate, Effects and Response

199 Modeling Oil Transport and Fate in the Beaufort Sea – Evaluation of Implications of Response Alternatives

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To address questions and support decision-making related to the potential fate of spilled oil in the Arctic and implications of applying potential response options, RPS ASA has modeled oil transport and fate for hypothetical spills in the Beaufort Sea using the SIMAP modeling system. Seasonal ice cover and movement information, simulated by coupled ice-ocean (hydrodynamic) models and from external remote sensing data sets, were utilized in SIMAP to simulate effects of oil encountering moving sea pack ice and fixed landfast ice, respectively. Oil moved with the ice, or was trapped in landfast ice, when ice cover exceeded 30%. The fate processes spreading, evaporation, volatilization, emulsification, and surface oil entrainment were modified by ice cover exceeding this threshold. Dissolution rates and dissolved aromatic concentrations were affected by the processes regulated by ice interactions. Alternative response options were simulated in summer open water and fall freeze-up conditions: no response and (highly effective) subsea dispersant injection. In the no-response case, surfaced oil became trapped in ice and was transported westward north of the Alaskan coast. Effective subsea dispersant use could eliminate this floating and ice-entrapped oil contamination, dispersing it into deep waters. Comparing the results assuming the two response alternatives, the water volumes affected by dissolved aromatics (>1 ug/l) are similar; however, the dose is about an order of magnitude higher in the dispersed-oil case, albeit much deeper in the water column. In most areas, the densities of biota would be expected to be higher in surface waters, as opposed to in deep waters where the subsea dispersant-treated oil would remain. Thus, while the toxicity of the deep-water contamination from the dispersant-treated case would be expected to be higher than that for the similar-sized affected volume of the no-response case, lower densities of biota at depth as compared to in surface waters, as well as protection of wildlife from oil and volatile emissions at the water surface, in/on ice and along shorelines, suggest that subsea dispersant use would lower the ecological consequences of such a catastrophic spill. However, the distributions and sensitivities of organisms would need to be examined for locations of concern before determining a specific response strategy and plan for a large blowout.

200 Biodegradation of Oil and Corexit 9500A by Arctic Marine Microorganisms

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Understanding the biodegradation of oil and the chemical dispersant, Corexit 9500A, in the Arctic becomes increasingly important in areas influenced by oil exploration. Biodegradation by indigenous microorganisms is thought to be the main process contributing to the removal of oil from the environment, and oil degradation potential has been documented in near-shore Arctic marine environments. Combining degradation rates with molecular microbial analyses can provide further insight into the identity of oil- and Corexit-degrading microbes and how the Arctic marine microbial community responds to oil and Corexit. We conducted incubation experiments with offshore and near-shore surface seawater collected in the Alaskan Arctic to quantify the biodegradation of either whole Alaska North Slope crude oil or the surfactant components of Corexit 9500A. Incubations were conducted at 2°C with minimal nutrient addition and sampled at 0, 5, 10, and 28 days for oil, and 0 and 28 days for Corexit incubations. Petroleum hydrocarbons were quantified using GC/MS and the surfactant components of Corexit 9500A were quantified by LC-MS/MS. Next-generation sequencing of 16S rRNA genes allowed the identification of bacteria that grew in response to either oil or Corexit 9500A. The GeoChip microarray was used to detect and quantify genes for oil biodegradation in mesocosm incubations over time. The indigenous microbial community degraded 30% and 40% of the crude oil within 10 and 28 days, respectively. In Corexit incubations, 77% of dioctyl sodium sulfosuccinate (DOSS) was biodegraded within 28 days. When Arctic seawater was incubated with either oil or Corexit 9500A, some bacterial taxa and oil biodegradation genes increased in their relative abundance, suggesting a potential role in biodegradation. Sulfitobacter, Polaribacter, Oleispira and Colwellia species increased in relative abundance over the course of the incubations in response to both oil and Corexit. Some of these genera were also active in the biodegradation of the Deepwater Horizon oil spill. Future studies are needed to better understand the seasonal and spatial variability of oil and Corexit biodegradation potential in Arctic seawater as well as in ice-covered waters.

201 Biodegradation of crude oil and impact on seawater and sea-ice arctic microbial populations following oil spill treatments in the Arctic

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The impact of a potential oil spill and response techniques on Arctic communities and the fate of the oil in this environment need to be better understood. The International Association of Oil and Gas Producers (IOGP) initiated the Arctic Response Technologies Joint Industry Program (JIP) and, as a part of an international team, is developing a Net Environmental Benefit Analysis (NEBA) support tool for decision-making and environmental impact assessments related to Arctic spills. The project presented here is part of this undertaking, and focusses on oil biodegradation and sea ice microbial communities. The emphasis is on better understanding the biodegradation process and microbial responses to petroleum, following different response technologies, through in situ-exposure experiments in Svalbard. The persistence and biodegradation of the different oil compounds in the sea ice and seawater were assessed following a simulated oil spill and different treatment scenarios. Total and active microbial populations in the different layers of the sea ice were characterized, their biodegradation capabilities evaluated and specific species and genes were quantified to evaluate the microbial response to oil spills and the inherent ability of indigenous microorganisms to degrade petroleum compounds. These results were combined with the oil chemical profile to (1) evaluate the fate of this oil in Arctic sea ice/water and (2) identify the best course of action to minimize negative impacts on the environment. The impact of spills and of different treatment scenarios (dispersant addition, in-situ burning, no treatment) through the ice layers down to the water column were studied using purpose-build semi-open mesocosm structures to perform realistic exposures in-situ. The characterization and, for some species, gene quantification showed surprisingly high bacterial numbers in pristine and contaminated sea ice. Analyses carried out on ice cores from oil-contaminated as well as subsequently treated sites suggest a change in microbial community structure, with an increase in psychrophilic alkane degraders.

202 Comparison of the Relative Sensitivity of Arctic Species to Oil and Dispersed Oil using Total Petroleum and PAH Measures of Toxicity

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Extended periods of open water have expanded the potential opportunities for petroleum and gas exploration and production in the Arctic, increasing the focus on understanding the potential impacts of released oil on aquatic organisms. In many cases, information regarding the toxicity of physically and chemically dispersed oil to Arctic species is generally limited and has been largely based on total petroleum hydrocarbon (TPH) measures of toxicity. In order to better understand the sensitivity of Arctic species to oil and chemically dispersed oil, the relative sensitivity of Arctic species was determined by their position within empirically derived species sensitivity distributions (SSDs) for a range of petroleum products. SSDs were generated using data for acute toxicity (LC50) derived only from spiked declining oil exposures to minimize between test variation. Toxicity data for 35 marine species were included in this evaluation, with a total of 8 Arctic species and 25 non-Arctic species tested in 26 different oil products. Toxicity data for TPH, naphthalenes, or total PAHs for similar oil product types (e.g., middle distilates, light or medium crudes) were grouped in the SSD analyses. Based on the available toxicity data Arctic species were found to have similar sensitivity as non-Arctic species when based on TPH or PAH measures of toxicity.

203 Transcriptomics identifies novel adverse outcome pathways from crude oil exposure in haddock early life history stages

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Crude oil spills are a worldwide ocean conservation threat. Fish are particularly vulnerable to the oiling of spawning habitats, and crude oil causes severe abnormalities in embryos and larvae. Full elucidation of adverse outcome pathways (AOPs) stemming from crude oil exposure of fish early life history stages is essential for better risk assessments relating to expansion of oil production intoNortheast Arctic regions such as the Lofoten archipelago in northern Norway. A key step towards this goal is an understanding of the underlying mechanisms for developmental defects arising from crude oil exposure. We have explored the transcriptional basis for four discrete crude oil injury phenotypes in the early life stages of the commercially important, Arctic-spawning species Atlantic haddock (Melanogrammus aeglefinus). These include defects in 1) cardiac form and function, 2) craniofacial development, 3) ion and water regulation, and 4) cholesterol synthesis and homeostasis. First, our findings identified a direct link between disruption of calcium-dependent contractility and cardiomyocyte proliferation though effects of oil exposure on key cardiac signalling molecules and transcription factors. These results thus provide the first clear molecular events linking disruption of excitation-contraction coupling by crude oil to altered cardiac morphology. Second, our data provide molecular insight into a complex pattern of genes involved in craniofacial skeletal and muscle development was consistent with an early effect on muscle development, secondarily disrupting the craniofacial cartilages. Finally, additional phenotypes relating to accumulation of oedema and shifts of body fluid and reduced yolk absorption were clearly linked to genes functioning in ion and water regulation and cholesterol biosynthesis. These latter findings suggest novel adverse outcome pathways resulting from crude oil toxicity in developing marine fish from high northern latitudes, and in particular identify cholesterol biosynthetic enzymes as potential bioindicators of oil-induced injury to fish embryos. Overall, our study clarifies initiating events for the well-established cardiotoxicity AOP, and identifies novel AOPs affecting Arctic-spawning Gadid fish. Future studies should characterize the effects of crude oil exposure on embryolarval osmoregulation and cholesterol synthesis at higher levels of biological organization.

204 An ecosystem-based modeling system for the prediction of potential impacts of petroleum and fisheries activities in the marine environment

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Fisheries and petroleum resource development are concomitant in some of the world's most productive continental shelf seas. Much of our understanding of the impacts of oil spills to fisheries and the general health of ecosystems has been acquired from a few major events. It remains a significant challenge to link data on effects of oil on individual organisms, mainly gathered through controlled laboratory experiments, to impacts on the population as a whole. The advanced software tool, SYMBIOSES, was developed to improve assessments of environmental impacts linked to oil spill scenarios, and in particular, effects of oil spills on fish stocks at the population level. The tool simulates the distribution and behavior of different life stages of important commercial fish species and their prey, effects of petroleum components on the eggs and larvae of fish and zooplankton, and multi-year changes in fish populations. It allows for impacts on larval survival to be traced through the population to observe the effect of oil spills on overall fish stocks. As a demonstration of the utility of such modeling tools, we will present simulation results for 38 oil spill scenarios, releasing 18-349 x 10^3 metric tons of oil from a single location on the northern Norwegian continental shelf (75.2N 32.4E). These release amounts are comparable to the 1989 Exxon Valdez oil spill in the Gulf of Alaska and the 1979 IxToc I oil spill in the Gulf of Mexico. We examine survival of early life stages of cod and the resulting impacts on the cod population. We will also demonstrate how SYMBIOSES may be used to assess two different oil spill mitigation strategies: reducing the fishing quota and applying dispersant. This study demonstrates the utility of predictive ecological models to explore and inform on a key impact factor of oil spills which, in turn, lends valuable support to science informed decision-making and stakeholder communication.

205 Estimated Impacts of Hypothetical Oil Spills in the Alaska Beaufort Sea on the Arctic Cod Boreogadus saida

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The effects of hypothetical spills of Alaska North Slope crude oil (ANS) on Arctic cod Boreogadus saida in the central shelf area of the Alaska Beaufort were evaluated using acute toxicity data; field studies of Arctic cod larval distribution and abundance; natural mortality estimates for Arctic cod eggs and larvae; an oil spill fate/effects model and a fecundity hindcasting biological model. Planktonic life stages of Arctic cod were considered the most susceptible to oil spill impacts. Multiple scales of spill events (1,000 tons, 10,000 tomns, 100,000 tons) were evaluated for both physically and chemically dispersed oil. The potential effects of accidental spills of dispersant only were also evaluated. A 100,000-ton spill of ANS treated with dispersants resulted in a toxic volume of 266 million m^3 as opposed to a volume of 71 million m^3 for a 100,000 ton spill of ANS not treated with dispersants. Dispersants rapidly transports large quantities of the oil from the surface into the water column. A 100,000-ton spill treated with dispersants resulted in estimated losses of about 2 million Arctic cod larvae remaining from an initial 87 million eggs. This level of egg produciton represents the reproductive output of about 7,300 adult females. The population of Arctic cod age 1+ has been estimated to range between 2 and 4 billion fish. These and other data presented herein suggest that adult Arctic cod (age 3) populations in the Alaska Beaufort number in the 10's to 100's of millions. For the time and place evaluated (mid-shelf Alaska Beaufort Sea, mid-August) even a large spill appeared unlikely to have the potential for major impacts on Arctic cod population.

206 Net Environmental Benefits Analysis Supporting Oil Spill Response in the Arctic

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Oil spill response in the Arctic is complicated by extreme environmental conditions, the presence of ice, and seasonally variable biological communities. Understanding the environmental consequences of different response strategies is critical to implementing actions that will minimize overall impacts of an oil spill event (either from the oil or response activities). The goals are to direct response decisions towards an optimum path for environmental recovery, and to choose options that promote long-term recovery. In a sensitive region such as the Arctic, these goals can be achieved using net environmental benefits analysis (NEBA) to compare and rank the consequences of different response alternatives and identify the potential long-term impacts of oil spill response options while accounting for changing Arctic conditions. NEBA is a transparent and science-based assessment process capable of incorporating a wide range of ecological and socioeconomic considerations. Weighting functions are used to place emphasis on the impacts to protected species and less resilient habitats. Statistical methods are used to quantify changes in ecological habitat values (e.g. fisheries habitat), social values (e.g. food resources) and economic values (e.g. commercial fish stocks) associated with different response options. This paper will review the use of NEBA in cold water environments to support both oil spill response planning and post-spill cleanup decision-making and discuss the results of recent work to enhance and advance the applicability of NEBA tools for oil spill response planning.

Recent Advances and Trends in Poly- and Perfluoroalkyl Substances Research – Part 1

207 Scratching the Surface of PFASs: An attempt at closing the mass balance using four techniques in select Papers & Textiles

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Per-and Polyfluoroalkyl substances (PFASs) have historically been characterized in numerous environmental matrices, however, only a small fraction of these compounds are included in current methods. This study used the most inclusive PFAS methods developed, and yet indications a number of unknown precursor compounds. Since PFASs' accumulation in blood and organs are due, in part, to exposure to consumer products, a small subset of seventeen papers and textiles were analyzed using four techniques: liquid chromatography tandem mass spectrometry (LC-MS/MS) for 73 individual PFASs, gas chromatography mass spectrometry (GC-MS) for 7 individual PFASs, total oxidizable precursor (TOP) assay, and total fluorine by particle-induced gamma-ray emission (PIGE) spectroscopy. The TOP assay was used to quantify the presence of precursors found in consumer products, but not represented by LC-MS/MS data by quantifying the formation of dead-end products including perfluoroalkyl carboxylates (PFCAs) and sulfonates (PFSAs). The net production of PFCAs/PFSAs that could not be accounted for as forming from identified individual PFASs was used as a measure of additional precursors. Nanomolar quantities of PFASs determined by LC-MS/MS and GC-MS and the TOP assay were compared for papers and textiles. In addition, TOP assay data were compared to the total fluorine content of papers determined by PIGE. Total PFASs measured by LC-MS/MS, GC-MS, and TOP assay accounted for less than 50% of the total PFAS present for the majority of these papers and textiles. To account for the missing mass, the extracted material (for LC-MS/MS) was analyzed through PIGE and showed agreement of total fluorine present in these materials, highlighting the utility of these assays for potentially obtaining mass balance on highly-fluorinated substances on papers and textiles.

208 Current Extraction Methods Significantly Underestimate Mass of Zwitterionic and Cationic Polyfluoroalkyl Precursors in Soil and Sediment

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Polyfluoroalkyl precursors, especially those with cationic functional groups, are likely to be sorbed to source zone soils and sediments where aqueous film-forming foams (AFFFs) were routinely used for fire-fighter training. AFFFs, containing per- and polyfluoroalkyl substances including polyfluoroalkyl precursors, were routinely applied at military and civilian sites. In order to characterize the sorption (partitioning) behavior of cationic and zwitterionic precursors, batch sorption experiments were conducted using a commercial AFFF formulation. After analyzing the aqueous, vial, and soil phases, the mass balance could not be closed using the current extraction method for polyfluoroalkyl precursors, which is based on low concentrations (15-20 mM) of ammonium hydroxide or sodium hydroxide in methanol. In order to close the mass balance, more rigorous extraction conditions were employed. The novel extraction method consisted of 0.5 M hydrochloric acid in methanol. With a more concentrated acidic methanolic extraction, mass balance was achieved for the cationic and zwitterionic precursors. The novel soil extraction method may reveal "additional" unreported mass of cationic and zwitterionic precursors in AFFF-impacted sites.

209 Qualitative and quantitative analysis of fluorosurfactants in soil impacted by aqueous film-forming foam after the Lac-Mégantic train derailment

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Aqueous film forming foams (AFFFs) rely on a mixture of hydrocarbon and fluoroalkyl surfactants (poly- and perfluoroalkyl substances, PFAS) to extinguish hydrocarbon fires by preventing fuel from contacting oxygen. The wide use of AFFFs for accidental fires or firefighting training has resulted in a high number of sites with elevated levels of PFAS. The recognition of the environmental and health impact posed by some PFAS, such as perfluorooctane sulfonate (PFOS), leads to the concern about the potential adverse impact of AFFF release. The town of Lac-Mégantic (Québec, Canada) was the location of a major deployment of AFFFs after the July 2013 train derailment accident. An estimated 33,000 litres of concentrated AFFFs were used to extinguish the fire from the spill of millions of liters of crude oil. Though a significant amount of AFFFs was recovered during the clean-up effort, some PFAS made their way into River Chaudière, the sewer system, and surrounding soil. Due to the proprietary nature of AFFF formulation, there is a lack of information regarding the nature of PFAS in AFFF formulation as well as their environmental fate, behaviours and impact. To fill the knowledge gap, we focused on PFAS identification in soil in the current study. Soil samples were collected days after the firefighting activities as well as two years later around the site of the accident. Soil from a biopile treatment system, where excavated oil-contaminated soils were subject to bioremediation, was sampled in 2015. A solvent extraction method was optimized for 34 PFAS analytes in the presence of hydrocarbons as co-contaminants. Lac-Mégantic soil samples were extracted using the method and analyzed both qualitatively and quantitatively using high resolution and tandem mass spectrometry systems. A suite of approximately 60 compounds was qualitatively detected, suggesting the possibility that the total PFAS contamination is significantly underestimated when only compounds for which standards are available are analyzed. Spatial and temporary profiles of PFAS will be presented along with the levels of co-contaminants, and the implications for future study on the PFAS originated from AFFFs will be discussed.

210 Newly-identified polyfluoroalkyl surfactants in the freshwater fish C. commersonii following AFFF deployment at the Lac-Mégantic railway accident

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On July 6th 2013, an unmanned train derailed downtown Lac-Mégantic (Québec, Canada), causing a spill of nearly 6 million liters of crude oil, destroying large parts of the city center, and claiming the lives of 47 people. In the emergency response to contain the fire, the deployment of aqueous film forming foams (AFFFs) lasted for approximately two days and > 30,000 liters of foam concentrate were applied. At least 4 out of the 7 firefighting formulations contained fluoroalkylated surfactants (PFASs), organofluorine chemicals whose persistence, bioaccumulation potential and adverse effects have been documented in the literature. The present study examines the environmental occurrence of historic and newly-identified PFASs in the benthic fish Catostomus commersonii and sediments collected from the adjacent Lake Mégantic and Chaudière River (downstream from the AFFF-impacted site). 28 target and 90 suspect-target PFASs were therefore investigated using ultra-high performance liquid chromatography polarity-switching electrospray ionization coupled to Orbitrap mass spectrometry. Seven zwitterionic or cationic PFASs were synthesized to provide for model analytes for method validation and semi-quantification purposes. In sediments, zwitterionic PFASs dominated PFAS composition profiles (~94%) while perfluoroalkyl acids (PFAAs) were less prevalent (~3%). Levels of PFAAs remained moderate in fish muscle (e.g., PFOS: 0.36–2.5 ng g^{-1} wet-weight), with little or no significant temporal trend when comparing 2013 or 2014 fish samples with 2011 archived samples. In contrast, fluorotelomer sulfonates peaked in the immediate weeks or months that followed the accident, as did several zwitterionic PFASs such as fluorotelomer sulfonamide betaines (8:2 FTAB, 10:2 FTAB) and fluorotelomer betaines (especially 9:3 FTB, 11:3 FTB, 7:1:2 FTB, 9:1:2 FTB and 11:1:2 FTB). With time, levels of betaine-based PFASs gradually decreased in fish, possibly indicating attenuation by biodegradation of the fluorine-free moiety, supported by the observation of likely FTAB metabolites such as fluorotelomer carboxylates and fluorotelomer sulfonamides. These metabolites may convert to PFAAs in the long-run.

211 Transport of Poly- and Perfluoroalkyl Substances in Groundwater, Cape Cod, MA

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Since the 1950s poly- and perfluoroalkyl substances (PFASs) have been used for many applications owing to their high stability and the combination of their surfactant, hydrophobic, and oleophobic properties. Groundwater PFAS contamination has arisen as a concern because of the widespread current and historical use of PFAS-containing aqueous film-forming foams (AFFFs) for fire suppression. Research is needed on PFAS transport properties in groundwater, as these contaminants pose a unique threat to groundwater quality owing to their persistence, bioaccumulation, ubiquity, and adverse human health effects. A site on Cape Cod, Massachusetts, where fire training activities involving AFFF were conducted from 1970 to 1985 has been investigated to understand the subsurface transport of PFASs. More than 100 groundwater samples were collected along a transect extending 1.1 km downgradient from the fire-training area and analyzed with LC-MS/MS to visualize the subsurface PFAS distribution along the groundwater flow path. Land disposal from 1936 to 1995 of secondary treated wastewater effluent onto infiltration beds located 500 m hydraulically downgradient from the fire training area resulted in a wastewater plume that comingles with the PFAS plume. PFAS transport properties were investigated upgradient of and within the wastewater-contaminated zone by comparing PFAS distributions to wastewater-affected geochemical parameters such as specific conductance, pH, temperature, and concentrations of dissolved organic carbon, dissolved oxygen, and dissolved iron. Perfluoroalkyl acids (PFAAs) with short chain lengths and PFAA precursors exhibit unexpected transport rates in the field when compared to results reported in the literature and laboratory partitioning experiments conducted with sediment cores from the field site. Distinctly different PFAS compositions in shallow groundwater below the vadose zones at the fire training area and wastewater-infiltration beds indicate that these are two compositionally different PFAS sources, suggesting that the PFAS composition in groundwater samples can be used to identify potential sources. This work contributes to the understanding of how PFASs migrate from point sources and identifies some mechanisms that could impact transport.

212 Perfluoroalkyl acid concentrations in historical drinking water samples collected from the Greater Cincinnati Region

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The Health Outcomes and Measures of the Environment (HOME) Study is an epidemiological study conducted in Greater Cincinnati area (Geraghty, et al., 2008), to examine the effects of low-level exposures to prevalent neurotoxicants in a cohort of 400 children followed from 16 weeks of gestation to 5 years of age. As part of the study, serum and drinking water samples were collected during 2003–2006 from each participant's home. Poly- and perfluoroalkyl substance (PFASs) concentrations were measured in serum samples of 6–8 year-old female participants (Pinney SM, et al. 2014). Elevated concentrations of perfluorooctanoic acid (PFOA) were found in 94% of the participants living in one area compared to the National Health and Nutrition Examination Survey (NHANES) 95th percentile for children 12–19 years (8.4 ng/mL), median 22.0 ng/mL. This was speculated to be derived from an industrial source upriver of the Ohio River through drinking water. Drinking water samples were collected directly from the taps into 500-mL HDPE containers and stored frozen until the time of lead analysis in 2007. The samples were transferred to smaller size tubes and stored again at –20 C until our analysis in 2016. The sample volumes were approximately 10 mL. We used a newly developed on-line solid phase extraction method to analyse PFASs in these drinking water samples. In a pilot scale analysis of 25 samples, median concentrations of PFOA and perfluorooctane-suflonic acid (PFOS) were 10 and 7.6 ng/L, respectively, which were comparable to the previously reported levels such as NHANES. The maximum levels for PFOA and PFOS were 108 and 98.6 ng/L, respectively. Some of the samples exceeded the newly established US Environmental Protection Agency (USEPA) Drinking Water Health Advisories for PFOA and PFOS (70 ng/L combined PFOA and PFOS). The full-scale analysis results for 400 samples will be reported and discussed in detail for the presentation. Disclaimer: The views expressed in this abstract are those of the authors and do not necessarily reflect the views or policies of the USEPA. The findings and conclusions of this article are solely the responsibility of the authors and do not represent the official views of the National Institute for Environmental Studies, Japan.

213 Perfluoroalkyl and polyfluoroalkyl substances associated with aqueous film forming foams in Canadian surface waters

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Dozens of novel perfluoroalkyl and polyfluoroalkyl substances (PFASs) have recently been identified in aqueous film forming foams (AFFFs), but environmental detections of these compounds are limited and they have not been previously detected in surface waters. Surface water samples were collected from rivers and lakes in Southern Ontario and Nunavut, including AFFF-impacted sites, urban rivers, and rural sites. Surface water samples were extracted by mixed-mode weak anion exchange solid

phase extraction in a clean lab and analyzed by UPLC-MS/MS both quantitatively where standards were available and semi-quantitatively for other AFFF components. Detection of AFFF components was confirmed by spiking surface water extracts with AFFF extracts. In terms of electrochemical fluorination products, concentrations of perfluorohexane sulfonate (PFHxS) approach those of perfluorooctane sulfonate (PFOS) in some AFFF-impacted waters, perhaps due to degradation of perfluorohexane sulfonamide (FHxSA)-based surfactants used in AFFFs, which were detected in some samples. FHxSA was detected in all AFFF-impacted and urban surface waters at concentrations from 0.04 to 20 ng/L, which indicates the relevance of PFHxS precursors in surface waters. Pre-PFOS phase out carpet protection products are also a potential source of FHxSA. Fluorotelomer PFASs were also frequently detected in surface waters, including 6:2 fluorotelomer sulfonate (FTSA, up to 3.0 ng/L), 6:2 fluorotelomer sulfonamide alkylbetaine (FTAB, up to 22 ng/L), 6:2 fluorotelomermercaptoalkylamido sulfonate sulfone (FTSAS-SO_2, up to approximately 1 ng/L), 6:2 fluorotelomerthioether hydroxyl ammonium sulfoxide (FTSHA-SO) and fluorotelomer betaines (FTBs). Sampling at multiple sites along the Welland River suggests that FTBs are particularly persistent fluorotelomer compounds. Relatively high concentrations of 6:2 FTAB in three watersheds in the Greater Toronto Area (3.5-18 ng/L) may imply other sources in addition to AFFF. Perfluoroalkyl carboxylate (PFCA) profiles of surface waters were generally dominated by C5 and C6 PFCAs over C7 and C8 PFCAs, which could reflect the switch from 8:2 fluorotelomer to 6:2 fluorotelomer PFASs in various applications.

214 Transport potential of per- and poly-fluoroalkyl surfactants in the presence of soil organic matter

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Repeated use of Aqueous Film-Forming Foam (AFFF) at fire-fighting training areas has resulted in groundwater pollution by a large number of per- and poly-fluoroalkyl substances (PFASs). Management and remediation of such contaminated sites require an understanding of the transport potential of these emerging pollutants. The mobility of perfluoroalkyl carboxylic acids (PFCAs) and sulfonic acids (PFSAs) is controlled by interactions with organic matter and hydrophobicity effect. For the short-chained acids, electrostatic interaction and steric effect appear to be important. As of today, little is known about the transport potential of other types of zwitterionic, cationic, and anionic PFAS, which due to their chemistry could behave very differently from PFCAs and PFSAs. In this study, interactions between 15 PFASs and model soil organic matter (SOM, Pahokee peat) were examined by a novel dynamic HPLC-based column method. The PFASs under examination include not only PFCAs and PFSAs but also fluorotelomer sulfonates (FTSs) and fluorotelomer betaines (FTBs), which have been detected at high levels in surface and ground water impacted by AFFFs. The PFAS elution profiles in the SOM-packed column were constructed through collecting HPLC eluent with the aid of a fraction collector and quantification by LC-MS/MS. Sorption coefficients normalized by organic carbon content (K_{oc}) were determined based on the retention volumes of the PFASs at three Ca^{2+} concentrations and over environmentally relevant pH range. At 5 mM $CaCl_2$, the observed $\log K_{oc}$ values of PFCAs (perfluoroalkyl carbon chain length, n = 3-8) and PFSAs (n = 4, 6, 8) were in the range of 1.27 – 2.53, which was largely consistent with literature values. The observed $\log K_{oc}$ values of FTSs (n = 4, 6, 8) were 1.32-2.48 and three FTBs were 1.84 – 2.20. Calcium ion has a positive impact on the sorption of anionic PFASs to SOM with $\log K_{oc}$ values increased by ~0.2-0.7 when Ca^{2+} concentration was rising from 0.5 to 50 mM. Moreover, raising pH slightly reduced sorption of all PFASs to the SOM. The $\log K_{oc}$ values were decreased by ~0.22-0.58 with pH increasing from 3.8 to 8. This phenomenon is likely caused by enhanced electrostatic repulsion between the anionic head group of PFASs and more negatively charged organic matter surface at a higher pH. Overall, sorption of these compounds follows a chain-length dependence trend. The results are useful for predicting transport behaviors of PFAS in AFFF-impacted sites.

Environmental Chemistry Perspectives from Around the Globe

215 Why TBT contamination still matters in South America

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Antifouling biocides contamination has been appraised along South American coastal areas during the last decade with most of these studies focused in butyltin (BT) levels and imposex occurrence (a known biomarker of TBT contamination). Due to many national wide use restrictions, and after the Antifouling System Convention (AFS) banning of TBT-based antifouling paints issued by the International Maritime Organization in 2008, environmental levels of TBT as well as imposex incidence begun to decline in many areas worldwide. In South America, TBT levels and effects also decreased in areas under the influence of commercial harbors. However, recent studies have pointed out that impacts related to TBT usage are still evident in sites of Argentina, Brazil, Colombia, Chile, Ecuador, Peru and Venezuela with maritime activities related to smaller vessels (mainly fishing and pleasure boats). In all cases, TBT concentrations detected were sufficiently high to induce environmental effects. Furthermore, recent TBT inputs have frequently been detected in many of these evaluations. This scenario is, at least partially, caused by the absence of local regulations on the use of TBT-based antifouling paints. Within South America, only Brazil and Argentina have some restrictions against TBT and only Brazil and Uruguay are signatories of the AFS convention. Even so, recent inputs have been found in two of these countries. In addition, TBT can be easily purchased by users for mixing to commercial antifouling paints to boost their efficiency. In addition, most antifouling paints marketed in South America do not include information on the active biocide used in their formulation, hindering legislative and oversight actions. Thus, the implementation of pertinent regulations, with special care about their effective application, is crucial to lower the inputs, and consequent impacts, of BTs on South American coastal areas as has happened in Europe and North America.

216 Trace Metal Pollution of Suburban Roadside Surface Soil by Road Dust

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Operation of motor vehicles is one of the major sources of environmental contamination, especially in urban environments. In many urban watersheds, tires and brake pads are significant sources of Zn and Cu, respectively. A previous study showed Houston road dust to contain trace metals at elevated concentrations. Houston road dust may be a significant source of trace metals to surrounding soil through atmospheric deposition and stormwater runoff. To better understand the fate and transport of trace metals in road dust, surface soil (2 cm) was collected 1 m from the edge of Highway 6 approaching a traffic signal in Sugarland, Texas at 200, 160, 100, 50, 25, 15, and 5 m from the intersection. Upon arrival at the laboratory, samples were dried, and sieved to fractions of 500 µm–1 mm, 250-500 µm, 125-250 µm, 63-125 µm, and2) are low for Cu (0.05) and Zn (0.003), and moderate for Cd (0.51), Ni (0.48), and Cr (0.53) indicating dissimilar sources. Copper and Zn likely originate from brake and tire wear particles, with deposition and resuspension being strongly influenced by wind and other vehicle-related forces. Significant fractions of Cd, Ni and Cr likely originate from concrete pavement wear. Relative anthropogenic metal input, known as enrichment factors (EF), is calculated using surface soil sample (< 63µm) collected on campus of Texas Southern University containing Cu (20.6 µg/g), Zn (104.9 µg/g), Cr (20.3 µg/g), and Fe (0.7%). Using Fe as the normalizing element, EF for these samples are Cu (1.7), Zn (1.5), and Cr (1.2). Due to limited uncertainty, EF values > 1 indicate moderate anthropogenic input, with surface soil being moderately polluted by Cu, Zn and Cr. Further studies will be completed to determine whether traffic-related trace metals are infiltrating roadside soil, or rather being transported through stormwater.

217 Platinum Group Elements in Bioaccessible Fractions of Street Dust from Toronto, Canada

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Road dust particles capable of resuspension are important contributors of metal contamination to local urban air and water sheds. Road dust has been shown to be enriched with potentially toxic metals such as cadmium (Cd) and lead (Pb). While platinum group elements (PGE), which includes platinum (Pt), palladium (Pd), rhodium (Rh) and iridium (Ir), are known to be accumulating in urban environments, less is known about their concentrations in road dust. This study addresses data gaps on environmental exposures to metals in an urban context, most notably those associated with bioaccessible road dust fractions. In the first study phase, PGE concentrations in coarse and fine fractions of road dust collected in Toronto were characterized to assess spatial variability as a function of road type and traffic volume. Samples were collected in Fall 2015 in cooperation with the City of Toronto, which uses regenerative street sweepers that dry-fractionate dust sweeps into coarse "hopper" (< 2 mm) and fine "dust box" (ca. < 3 microns). PGEs were determined in air-dried, sieved samples using NiS fire-assay-INAA. The results confirm an enrichment of Pd, Pt, Rh and Ir in road dust, attributed to emissions from automotive catalytic converters. Overall, Pd was observed to occur at the highest relative concentrations in road dust, with a City median of 115 µg/kg, followed by Pt (median: 52 µg/kg) and Rh (median: 15 µg/kg) and Ir (median: 0.40 µg/kg), while Os and Ru were below limits of detection. Pd concentrations were the most variable, ranging from a low of 16 µg/kg (local/residential road) to a maximum of 344 µg/kg (municipal expressway). Fine/coarse enrichment ratios for PGE increased from lowest to highest in the following order: Pt(2)=Pd(2)< Rh(3)< Ir(10). The observed two-fold to ten-fold enrichment of PGE in the finer fractions of road dust is of concern, given the greater relevance of the fine fraction to environmental and human health. The preliminary results of this study indicate a need to more closely examine the concentrations of potentially toxic elements associated with fine road dust fractions. Next steps will involve the elemental characterization of sweeps collected in Spring 2016 in the City of Toronto and the application of in vitro methods to assess the chemical bioaccessibility of metals in road dust.

218 Biogeochemical processes occurring in forested mountain catchments deduced from chemical and sulfur isotopic composition of drainage water

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Physical catchment features influence the chemical and isotopic composition of their drainage water (streams and seeps). Lower stream orders by definition integrate smaller drainage areas; thus, the biogeochemical processes occurring in forested mountain catchments are reflected most clearly in first-order streams and interflow seeps (i.e., consisting of recent precipitation). In the Great Smoky Mountains National Park (GRSM), grab samples of first-order stream, tributary and seep water were analyzed for a suite of chemical parameters (e.g., pH, acid-neutralizing capacity [ANC], and dissolved ions) and sulfur isotope composition. A wide range of drainage water compositions were identified (low and high sulfate content; low and high pH and ANC), but high sulfate concentrations were not consistently associated with low pH/ANC. Spatial distribution of drainage water with a given composition was 'patchy:' catchments with low pH/ANC drainage water were commonly found adjacent to those with moderate to high pH/ANC drainage water. Drainage water composition resulting from interaction with sulfidic bedrock can appear similar to that resulting from deposition of acid-forming atmospheric sulfur oxides. However, the water composition of streams and seeps sampled in this study was not related to elevation (as expected if atmospheric deposition was the dominant source of sulfur and acidity), or to reductions in deposition of atmospheric sulfate. It did show some relationship to catchment geology, specifically to the extent of exposed sulfidic bedrock. This drainage water composition survey demonstrates the relative importance of catchment geology in driving biogeochemical processes that influence the character of catchment drainage water, and shows how drainage water composition can be used to deduce catchment-specific characteristics.

219 Colloidal-facilitated transport of nitrogen as a function of land-use in a major river basin discharging to the Great Barrier Reef lagoon

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Since European settlement of Australia, sediment and nutrient loads to the Great Barrier Reef (GBR) marine zone have increased dramatically as a result of land-use changes, particularly agricultural intensification. The resulting reduction in water quality has been linked to eutrophication, loss of coastal marine biodiversity and a dramatic reduction in GBR coral cover. Recent research has suggested that up to 87-95% of sediment < 10 µm in diameter exported from the Burdekin River Basin into the GBR lagoon is the result of the erosion of gullies, which are common landscape features in areas used for grazing. Fine sediment can directly reduce coastal water clarity and coral recruitment, while N has the potential to be transported over a much larger area and further reduce water clarity by feeding primary production. The degree to which this subsurface sediment loss is comprised of mobile colloids is unknown, as is the degree to which these colloids may facilitate transport of nitrogen into the GBR lagoon. Here we examine water-dispersible colloids (WDC) collected from areas under sugar cane and compare them to WDC collected from gullies formed in grazing lands. Physical characteristics of WDC were assessed via measurement of particle size/morphology, mineralogy and electrophoretic mobility. Total N concentration associated with the WDC as well as with < 0.45 µm and < 3kDa size fractions were analyzed. As ^{15}N abundance in N from animal waste is distinct from that of N in inorganic fertilizer, the ^{15}N natural abundance in the WDC and in the < 3kDa size fraction were assessed to determine if colloid-associated N can be linked to specific land uses. We anticipate that the results of this research will help refine our understanding of N fluxes into the GBR lagoon and will contribute to the development of improved models and land management practices.

220 Occurrence of brominated and organophosphorus flame retardants in indoor dust in a Brazilian city

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Indoor dust is indicated as one of the most important human exposure routes to flame retardants (FRs). Most of data about occurrence and levels of FRs in dust are from North American, European and Asian Countries, but scarce data is available for countries that do not have regulatory laws concerning fire safety of materials, as is the case of many South American countries. In this study, eight polybrominated diphenyl ethers (PBDEs), four new brominated flame retardants (NBFRs) and ten organophosphorus flame retardants (OPFRs) were determined in indoor dust from different places in Araraquara-SP (Brazil). A total of 46 samples were collected from homes (houses and apartments), workplaces, primary schools and cars. OPFRs, PBDEs and NBFRs were detected in 100% of the samples. OPFRs were present at the highest concentrations, and the most abundant compounds were TBOEP, TPHP, TDCIPP and TCIPP. The most abundant brominated FRs were BDE-47, BDE-99, BDE-209, BEH-TEBP and DBDPE. It was hypothesized in this study that soil particles blowing into the indoor environment from outdoors could have a significant dilution effect on the FR levels in house dust, but it was not confirmed since no difference in terms of concentration was observed among house and apartment samples. In addition, the FR distribution was also similar among houses and apartments, and were correlated to the presence of electronic equipments, furniture and construction materials present in each sampling site. Primary schools presented FR levels similar/lower than the observed in homes, except for TBOEP, that was about thirty times higher in schools. Workplaces presented the highest FR levels, mainly for brominated FRs, attributed to the presence of several computers and electronic equipments in these places. Most of FRs were detected in car dust samples, and TDCIPP, TPHP, BDE-209, BEH-TEBP and DBDPE were the most abundant compounds. An OPFR increasing tendency associated to a PBDE decreasing was observed for newer vehicles, indicating that international prohibition on PBDEs use is also influencing Brazilian auto industry. In conclusion, FR levels and profiles in this study were similar to the observed in other countries, indicating that although Brazil do not regulates the use of FRs, these compounds are present in the commercialized products, which are diffusive sources of FRs to the surrounding environment, exposing humans to these contaminants.

221 Evaluating Primary Contaminant Pathways to Combined Sewer Systems Discharging to the Lower Duwamish Waterway Superfund Site, Seattle, WA

C. Greyell, D. Williston, R.A. Jack, J.H. Stern, King County / Dept of Natural Resources and Parks

King County conducted a pilot study to examine pathways of contaminant sources to combined sewer basins that discharge to the Lower Duwamish Waterway Superfund site in Seattle, Washington. In combined sewer basins, both sewage (domestic and industrial wastewater) and stormwater are normally routed to wastewater treatment facilities. However, during large storm events, these basins can temporarily route excess sewage and stormwater directly into nearby waterways as combined sewer overflows (CSOs) to prevent sewer backups into homes and streets. Because CSOs can be a pathway of contamination to the waterway, understanding whether contaminants mainly enter the system via sewage or stormwater can help guide source control activities in the combined sewer basins, which supports the Superfund sediment cleanup effort. From 2011 to 2013, aqueous samples were collected with autosamplers from two combined sewer systems along the Lower Duwamish Waterway during both baseflow (sewage only) and stormflow conditions (sewage and stormwater). Samples were analyzed for metals, PAHs, phthalates, PCBs, and dioxin/furans. Flow was also measured to calculate the average flow rate during each sampling period. Average chemical loading rates were then estimated for each flow condition to evaluate the primary pathway (sewage or stormwater) for each contaminant during storm events. Stormwater was identified as the primary pathway for most contaminants in each study basin, despite differences in land-use. The findings of this pilot study can be used by King County to guide basin-wide source tracing efforts in combined basins prior to CSO control.

222 Pharmaceuticals, plasticisers, perfluorinated compounds and illicit drugs in the aquatic environment: What is their fate?

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Pharmaceuticals, personal care products (PPCPs) and other emerging contaminants (ECs) such as plasticisers, perfluorinated compounds (PFCs) and illicit drug metabolites are known contaminants of the aquatic environment. Most commonly, the study of such compounds is limited to their occurrence dissolved in river, lake or ocean waters. Few studies assess their accumulation in suspended particulate material, sediment, and aquatic organisms. Here, thirteen markers of anthropogenic contamination were selected for the study of their occurrence in the aquatic environment. PPCPs/ECs studied included pharmaceuticals acetaminophen, diclofenac, and ethinylestradiol, plasticisers bisphenol-A, bisphenol-S and 4'-hydroxyacetophenone, perfluorinated compounds (PFCs) perfluorooctanoic acid, perfluorooctanesulfonic acid, perfluorononanoic acid and perfluorobutane sulfonic acid, and illicit drugs/metabolites methamphetamine, amphetamine and benzoylecgonine. Selected contaminants were quantified in river water (n=69), sewage treatment works (STW) effluent (n=22), field/street drainage (n=5), bound to SPM (n=16), river sediment (n=23), bioaccumulation in aquatic plants Callitriche sp. (n=8) and Potamogeton sp. (n=7), amphipod crustacean (Gammarus pulex, n=10) and the aquatic snail (Bithynia tentaculata, n=7). Solid phase extraction followed by an in-house validated method for liquid chromatography-tandem mass spectrometry was used for all analysis. Extractions were carried out using ultrasonic-assisted solvent extraction. Highest levels of pharmaceuticals were recorded in STW effluent and typically did not exceed 1 ug/L while levels of plasticisers and PFCs were generally higher in street runoff (typically not exceeding 2 ug/L). Accumulation was lowest in sediment and highest in Callitriche sp. where levels of target contaminants typically did not exceed 75 ng/g dry sediment and 250 ng/g dry plant respectively. Here we demonstrate that quantification of PPCP/EC occurrence in river water alone is not a sufficient assessment of these contaminants in the aquatic environment. The widespread occurrence of selected contaminants in this study highlights the importance of less well-characterised means of exposure to such contaminants (e.g., sediment and food sources for higher organisms). To the knowledge of the authors, this is one of, if not the most detailed accounting of PPCP/EC fate and distribution in the aquatic environment conducted in the United Kingdom to date.

Alternative Approaches to Animal Testing for Ecotoxicity Assessments – Part 1

223 Quantifying the Benefits of Using Read-Across and In Silico Techniques to Fulfill Hazard Data Requirements for Chemical Categories

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Substantial benefits are realized through the use of in silico techniques to fill data pags for structurally similar substances. Considerable experience in applying these techniques was gained under two voluntary high production volume chemical (HPVC) programs – the International Council of Chemical Associations' (ICCA) (with the cooperation of the OECD) Cooperative Chemical Assessment Programme and the USEPA's HPV Challenge Program. These programs led to the compilation and publicly availability of baseline sets of health and environmental effects data for thousands of chemicals. The American Cleaning Institute's (ACI) contribution to these national and global efforts included the compliation of these datasets for 261 substances. Due to the structural similarity of the chemicals within the categories, their environmental fate, physical-chemical and toxicological properties are likely to be similar, which was confirmed by examining available data from across the range of substances in a category. This has allowed the utilization of read-across and trend analysis techniques and qualitative structure activity relationship (QSAR) tools to fill data gaps. Quantifying benefits resulting from avoided testing through the use of in silico tools, namely, the associated avoidance of testing costs and sacrifce ofanimals lead to estimates that, for these 261 substances, the use of tens of thousands of test animals and expenditures of millions of dollars were avoided.

224 Balancing the effectiveness and practicality of alternative test endpoints for the fathead minnow fish embryo toxicity test

[illegible], Texas Christian Univ / Biology; [illegible], Texas Christian Univ; [illegible], M.K. Sellin Jeffries, Texas Christian Univ / Dept of Biology

In recent years the fathead minnow fish embryo toxicity (FET) test has been developed and proposed as an alternative to the larval growth and survival (LGS), a whole effluent toxicity test utilized in the United States. The FET test represents a positive step forward with regard to the welfare of fish used in toxicity testing; however, the utility of the FET test is limited due to its reliance on lethal endpoints. Here, we sought to determine whether the inclusion of sublethal metrics could enhance the FET test by improving test sensitivity or by allowing for the prediction of adverse sublethal or chronic effects. FET tests were conducted using three reference toxicants—sodium chloride, ethanol and sodium dodecyl sulfate. Typical FET test endpoints (i.e., coagulation, presence of heart beat, somite formation and tail detachment) were evaluated in addition to three sublethal endpoints – wet weight, the presence of developmental abnormalities and the expression of genes associated with growth, stress and toxicity. Significant reductions in growth were observed in FET tests conducted with NaCl and EtOH, albeit only among embryos exposed to the highest concentration of each chemical. Pericardial edema was the most commonly observed developmental abnormality and was found to respond to each of the chemicals in a dose-dependent manner. Alterations in expression of a few genes related to growth and stress (i.e., igf-1, ghr and hsp70) were also noted; however, such changes were chemical specific and tended to only occur among embryos exposed to chemical concentrations also capable of eliciting significant mortality. An evaluation of each of these endpoints with regard to their practicality for FET test use (i.e., cost, time required and experience required) suggests that growth and edema could serve as potential FET test endpoints, while gene expression may not be as practical given the resources required for its assessment.

225 Alternative Testing Platforms for Screening Ecological Hazards

C. Lehman, Dow Chemical Company / Toxicology and Environmental Research and Consulting

We have developed and validated small scale, screening-level, environmental toxicity assays for fish, daphnia and algae for use in our laboratory. These assays are typically conducted in a well-plate format, and they have reduced cost and animal use over traditional full-scale OECD or OCSPP guideline tests. The fish screening assay utilizes fish embryos in lieu of juvenile or adult fish, thereby reducing the number of animals used for testing, aligning with the 3 Rs of animal testing (refine, reduce, replace). Using the three screens to determine which taxon is the most sensitive may further reduce the need to test fish species altogether. The screens can also be used to develop Predicted No Effect Concentrations for screening-level risk assessments. These small scale screens have business utility as well. Often during product development limited quantities of test material are available, and these screens conducted in well-plates can significantly reduce the amount of test material required for exposures, which reduces cost and waste. Since many products that chemical companies develop are novel chemistries, they are not always within the domain of accepted quantitative structure activity relationships (QSARs) or other predictive models. Thus, these screening level environmental toxicity assays can provide critical toxicity information early in the process of identifying candidate chemistries for further development. Frequently, product registration packages include read-across arguments among chemical categories, which assume the ecotoxicity of a molecule is comparable to other chemicals with similar structures. Strategically filling in gaps or uncertainties in read-across arguments or checking the reliability of QSAR models is also a potential use for these screening assays. Despite the many advantages of these assays, only traditional assays are recognized as reliable in compliance testing. We propose that these high-throughput assays gain regulatory acceptance by outlining their utility, versatility, and potential compliance advantages.

226 Possibilities for Using Fish Embryo Tests in Place of Fish Acute Toxicity – Threshold Approach Strategies for Ecotoxicity Hazard Determination

S.E. Belanger, J.M. Rawlings, The Procter & Gamble Company / Environmental Stewardship and Sustainability

The Threshold Approach is a sensible strategy used by ecotoxicologists to prioritize chemicals for testing using fish as described in OECD Guidance No. 126. Acute testing is initially conducted using algae and Daphnia and the lower of the two values is used to set a limit test on juvenile fish acute toxicity testing (OECD 203 TG). If fish display no mortality, a decision is made that the lower tested value from algae and Daphnia can be used for hazard classification and risk assessment purposes in the absence of a full 96 hr LC50 study on vertebrates. Large savings in animal testing is the result. With the advent of Fish Embryo Tests (OECD 236 TG) we have re-evaluated the Threshold Approach in light of replacing acute fish tests with fish embryo tests. A data base on comparative FET-fish acute toxicity from Belanger et al. (2013) was extended to include algae and Daphnia in order to address the question if similar conclusions on the utility of the Threshold Approach would be expected if full replacement occurred. A total of 166 comparisons of Fish and FET toxicity were compiled (updating Belanger et al. 2013) of which 79 compounds were found to have a full complement of FET, fish, algae, and Daphnia data. An additional 128 compounds had FET, fish and Daphnia data (no algae) and 87 compounds had FET, fish, algae (no Daphnia). In this talk we will review the chemical coverage for each data set and the relative rankings of acute toxicity based on geometric mean and lowest available aquatic toxicity values in order to assist a judgement of the appropriateness of the FET to be used as a replacement for fish in the Threshold Approach Guidance. The expectation, given the FET-fish acute toxicity relationship of Belanger et al. (2013) and Busquet et al. (2014) is that the FET provides equivalent information to that of fish in this process. Recommendations for OECD, based on our findings, will be provided.

227 Mode of Action (MOA) Assignment Classifications for Ecotoxicology: Evaluation of Available Methods

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There are various structure-based classification schemes to categorize chemicals based on MOA which have been applied for both eco and human toxicology. With increasing calls to assess 1000s of chemicals, some of which have little available information other than structure, understanding how each of these MOA schemes was devised, what information they are based on, and the limitations of each is critical. Several groups are developing low-tier methods to more easily classify or assess chemicals, using approaches such as the ecological threshold of concern (eco-TTC) and chemical-activity. Evaluation of these approaches and determination of their domain of applicability is partly dependent on the MOA classification that is used. The objectives of this study were to evaluate available MOA classification methods using a set of unique chemicals from a large aquatic toxicity dataset, compare the various approaches, and evaluate their utility and limitations in low-tier assessment approaches. The most commonly used MOA classification schemes for ecotoxicology include Verhaar and Russom (included in ASTER), both of which are used to predict acute aquatic toxicity MOA. Verhaar is a QSAR-based system that classifies chemicals into one of 4 classes, with a 5th "other" class, whereas ASTER/Russom includes 8 classifications. Other methodologies include Toxicity Estimation Software Tool (TEST), an application that allows prediction into 5 broad MOAs, with specific MOAs as subcategories. MOAtox is a dataset of MOA assignments for >1200 chemicals that draws from various schemes and assigns 6 broad and 31 specific acute aquatic toxicity MOAs. Other classification systems that can provide insight on a chemicals' MOA include ECOSAR, EPISuite, and the OECD QSAR Toolbox. In this study, >5600 unique CAS numbers were evaluated using the classification and MOA schemes described above. In all of the approaches used, a large percentage (>40%) were classified as narcotics, with very in categories describing more specific mechanisms. However, a large percentage of chemicals were not classified for various reasons. This presentation will provide an overview of available MOA assignment tools, including summarizing their technical basis, comparing consistency in MOA classifications, and assess utility for grouping compounds in large structurally diverse datasets. This presentation does not necessarily reflect the views or the policies of the USEPA or European Commission.

228 Ecological Threshold for Toxicological Concern (eco-TTC) – Assessing the Potential of a New Tool for Environmental Hazard Assessment

A. Beasley, The Dow Chemical Company / TERC; M.G. Barron, USEPA / Gulf Ecology Division; S.E. Belanger, The Procter & Gamble Co. / Central Product Safety Dept; J. Brill, The Procter & Gamble Co. / Environmental Stewardship and Sustainability; D. DeZwart, DdZ-Ecotox; M.R. Embry, B. Farr, ILSI Health and Environmental Sciences Inst (HESI); M. Halder, A. Kienzler, European Commission Joint Research Centre / IHCP EURL ECVAM; T.J. Norberg-King, USEPA / NHEERL / Mid-Continent Ecology Division; H. Sanderson, Aarhus Univ / Environmental Science; R.M. Sternberg, USEPA / Environmental Fate and Effects Division; P.W. Wilson, Sanofi US, Inc. / Health Safety and Environment

The Threshold for Toxicological Concern, or TTC, is well-established for assessing human safety and has been applied for a variety of endpoints including carcinogenicity, teratogenicity, and reproductive toxicity. Recently, we have proposed an extension to the human safety TTC concept for application in environmental situations, termed the ecological TTC or eco-TTC. Eco-TTCs summarize the wealth of ecotoxicological information as Predicted No-Observed Effect Concentrations (PNECs) on diverse chemical substances in the form of statistical (probability) distributions. Eco-TTCs can be developed that allow prediction of untested chemicals based on structural attribute (category), mode of action, or functional use. The approach may be useful for assessing chemicals at early tiers of the risk assessment process, providing hazard perspective on chemicals that lack QSARs, guiding product development discussions, and assisting read across or category justifications. The eco-TTC approach has the potential to reduce the need for vertebrate testing (e.g., fish) in many situations. A database consisting of approximately 110,000 unique ecotoxicological records has been developed based on recent assessments of published data and international chemical management programs. This toxicity data is associated with physical chemistry data and curated taxonomic information for the organisms tested. A process to conclude acute and chronic effects as well as identify the PNEC for exposed ecosystems based on depth and breadth of data have been devised, with the 5th percentile of PNECs for a compound group defined as the ecological TTC. Several mode of action schemes are being assessed to devise a best approach for grouping compounds. Chemicals that are categorized as neutral organics are the most abundant in the dataset, therefore are candidates for an initial in-depth assessment of eco-TTC attributes. Approximately 500 chemicals in the database are included in this analysis at this time with approximately one third having complete acute or chronic data sets (all three taxa). The eco-TTC for non-polar and polar narcotics is explored in depth, with additional categories under development (phenols, esters, reactive compounds, surfactants, pesticides, and pharmaceuticals). Eco-TTCs look to be a promising addition to the toolkit of hazard assessment. This presentation does not necessarily reflect the views or the policies of the USEPA or European Commission.

229 Animal Alternatives for Whole Effluent Toxicity Testing: Perspectives from a Global Workshop

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Since the 1940s, effluent toxicity testing has been utilized to varying degrees in many countries to assess potential ecological impacts and assist in determining necessary treatment options for environmental protection. However, it was only in the early 1980's that toxicity based effluent assessments and subsequent discharge controls became globally important, when it was recognized that physical and chemical measurements alone did not protect the environment from potential impacts. Consequently, various strategies using different toxicity tests, whole effluent assessment techniques (incorporating bioaccumulation potential and persistence) plus supporting analytical tools have been developed over 30 years of practice. Numerous workshops and meetings have focused on effluent risk assessment through ASTM, SETAC, OSPAR, UK competent authorities, and EU specific country rules. Concurrent with this drive to improve effluent quality using toxicity tests, interest in reducing animal use has risen. The Health and Environmental Sciences Institute (HESI) organized and facilitated an international workshop in March 2016 to evaluate strategies for concepts, tools, and effluent assessments and update the toolbox of for effluent testing methods. The workshop objectives were to identify opportunities to use a suite of strategies for effluents, and to identify opportunities to reduce the reliance on animal tests and to determine barriers to implementation of new methodologies. As preparation for the workshop, a survey was conducted to assess the use and application of ecotoxicity-based effluent assessments. Preliminary results from this ongoing global survey of effluent assessment strategies were presented in May 2016 at SETAC Europe (Nantes). This talk will focus on the highlights of the workshop, including a review the state of the science, description of the suite of strategies being used for reducing the impact of effluents, discussion on ways to integrate alternative approaches, and identification of opportunities to lessen the reliance on animal testing. Finally, we will give an overview of how various countries approach effluent testing and discuss how these approaches and data generated can integrate into existing risk assessment methodologies. DISCLAIMER: The views, conclusions and recommendations expressed are those of the authors and do not necessarily represent the policies or positions of the European Commission, Canada or USEPA.

230 TSCA Reform: New Options for Animal Testing Alternatives

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The Frank R. Lautenberg Chemical Safety for the 21st Century Act (Lautenberg Act) will change how chemicals are regulated in the United States. The Lautenberg Act, which reforms the Toxic Substances Control Act (TSCA), requires more careful control and more thorough understanding of hazardous chemicals in the US marketplace. This is consistent with many global regulatory reforms that have already been implemented or are underway. Though many details of the Lautenberg Act and its implementation by the United States Environmental Protection Agency (USEPA) remain to be elucidated, some broad changes to chemical hazard data requirements are expected. In particular, the Act will grant USEPA expanded authority to require companies to obtain additional chemical hazard data. In the backdrop of what are likely to be significantly increased data needs, USEPA is simultaneously stressing that companies should use alternative test methods and tiered testing plans to reduce vertebrate animal testing, if possible. There is a global trend toward increased use of alternative sources of hazard information, including in vitro methods, high-throughput screening, in silico methods such as quantitative structure activity relationships (QSARs), and chemical grouping and read-across approaches. As part of the reforms enacted by the Lautenberg Act, USEPA will be required to develop a strategic plan to encourage the use of these alternative information sources. This presentation will highlight the new possibilities for taking advantage of these approaches, as well as some of the technical challenges that are likely to be encountered during the implementation of the new regulation.

Modeling and Monitoring of Environmental Pesticides Exposure: Regulatory Context and Improvements from Science

231 Plant Uptake – From Scientific Description to Regulatory Implementation

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Plant uptake is a crucial process for a comprehensive leaching assessment of pesticides and their metabolites and a realistic description of plant uptake can be critical for passing the regulatory groundwater exposure assessment. The process of plant uptake is described by parameters like plant uptake factor, transpiration stream concentration factor and root concentration factor. The science behind these parameters, experimental methods of their determination and the consistent and coherent use in leaching models is discussed. Furthermore, an overview on current regulatory guidance, practice, und issues is given. Proposals for use of plant uptake in regulatory leaching assessments should consider appropriate endpoints derived from robust laboratory studies as higher tier option, which provides for a pragmatic approach but protective assessment.

232 Modelling the Fate of Pesticides in European and French Cropping Systems: Integration of Complex Cropping Practices in the Regulatory Risk Assessment

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Modelling the fate of pesticides in the environment can help preventing and managing soil and water contamination. In particular, groundwater protection is a key issue for human health and resources sustainability. At the European level, a pesticide shall only be approved if the predicted concentrations in groundwater (PECgw) of the pesticide or of relevant metabolites are below 0.1 µg/L. The PECgw have to be estimated using at least two of four recommended models (MACRO, PELMO, PEARL, PRZM) together with representative European agro-pedo-climatic scenarios available for each crop (FOCUS scenarios). These scenarios assume that the same crop is grown every year. This assumption can be considered as a worst case for PECgw assessment but it does not include crop rotation. The PECgw have to be lower than 0.1 µg/L for at least one of the scenarios for pesticide approval. At the French level, there are two levels of risk assessment for plant protection products (PPP) containing the approved pesticide(s). First, PECgw are estimated from the FOCUS scenarios. The PECgw have to be lower than 0.1 µg/L for all representative scenarios. If not, a refined level of assessment is required. One refinement is based on numerous French agro-pedo-climatic scenarios (FROGS scenarios) coupled with the PEARL model. In contrast to FOCUS scenarios, FROGS scenarios allow to define risk mitigation measures based on specific cropping practices (i.e crop rotation). In order to be as representative as possible of standard agricultural practices, typical crop rotations of 31 French agricultural regions are implemented in FROGS. PPP can only be approved for conditions where PECgw are lower than 0.1 µg/L. However, while these scenarios consider more realistic cropping systems than the FOCUS scenarios, they do not take into account agricultural practices such as cover crops, mulch… In addition, the representation of agricultural practices in the numerical models remains incomplete. Therefore, new tools are needed to consider the variability of cropping systems in risk assessment. Among the most recent developments, the sequential use of a pesticide fate model such as MACRO and a crop model able to simulate the growth of a large range of crops under different agricultural practices (residues management, tillage...) is a promising method to refine risk assessment of pesticides. In the future, it would allow to consider more realistic cropping systems for pesticide regulatory risk assessment.

233 Assessment of available monitoring data and modeled EEC values for an herbicide (linuron) in the US and Canada

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The purpose of his study was to compile a dataset of monitoring data for an herbicide (linuron) used in North America and to create a geospatial illustration of the water monitoring locations, and to compare these results to the outputs of standard Tier 2 models. Water quality monitoring records were collected for a 25-year period (1986 – 2015). Over 115,000 sample results were obtained for Canada and the US. Monitoring data for the U.S. was available primarily online. Much of the information for Canada was requested from regulatory agencies and other organizations, and, to a lesser degree, available online. Of the 115,000+ records that were collected 89% are associated with the US and 11% of the monitoring data is for Canada. A majority of the data in both the Canada and the US are for surface water (~60%) and for groundwater (~39%), while results for drinking water are less commonly reported (0.02%). About 1% of the monitoring data indicated that the herbicide was detected. In addition to standard percentile assessments, geo-spatial statistics were applied to identify ranges of sampling density for monitoring and to determine if spatial-temporal trends are present in the data. Monitoring data were compared to standard USEPA and PMRA modeling scenarios. The results indicated that no correlation exists between modeled data and observed data for linuron.

234 Insecticides in sediment cores from a rural and a suburban area in South China: A reflection of shift in application patterns

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A shift in pesticide application pattern has occurred in recent decades, yet little information is available in the consequence of this shift. To better understand how the shift is reflected in aquatic environment, two sediment cores were collected from a rural (RLY) and suburban (SGZ) river in South China. A variety of legacy organochlorine pesticides and current-use pesticides (CUPs), including organophosphate, pyrethroid and phenylpyrazole insecticides were quantified at distinct increments of the sediment cores. Total insecticide concentrations were in the ranges of 67.6–1671 and 99.2–231 ng/g dry weight in RLY and SGZ with pyrethroids and organochlorines being the dominant components, respectively. In general, the shifting profile of sediment-bound insecticides from legacy organochlorines to CUPs over time followed their historical application pattern, but significant differences were noted between the temporal profiles of the rural and suburban cores in regards to concentrations and composition of insecticides. The observed difference between the suburban and rural cores was synchronous with land use pattern and local economic changes. A steep increased occurrence of CUP in the 1990s was observed in the RLY core, which is consistent with the onset of economic growth in this area. In contrast, the suburban SGZ area has been historically contaminated by legacy OCPs, with fresh input of OCPs in SGZ believed to be caused by soil erosion, caused by land reclamation activities associated with urban expansion. The current study shows the shift in insecticide application pattern from legacy organochlorine to CUPs leading to an elevated CUP occurrence in the environment. It also suggests a stronger need for understanding not only environmental fate and risk, but also how their use pattern and land use changes impact the occurrence of pesticides.

235 Chlorpyrifos in Surface Waters of Central Valley, California: Statistical Analysis of Environmental Monitoring Data

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Pesticides are routinely monitored in surface waters and resultant data are analyzed to assess whether their uses will damage aquatic eco-systems. However, the utility of the monitoring data is limited because of the insufficiency in the temporal and spatial sampling coverage and the inability to detect and quantify trace concentrations. This study developed a novel assessment procedure that addresses those limitations by combining 1) statistical methods capable of extracting information from changing detection limits, 2) statistical resampling techniques that account for uncertainties rooted in the non-detects and insufficient sampling coverage, and 3) multiple lines of evidence that improve confidence in the final conclusion. This procedure was demonstrated by an assessment on chlorpyrifos monitoring data in surface waters of California's Central Valley (2005–2013). We detected a significant downward trend in concentrations, which cannot be observed by commonly-used statistical approaches. We assessed that the aquatic risk was low by using Joint Probability Curve that works with non-detects and can differentiate indicator groups with varying susceptibility and recovering ability. In addition, we showed that the frequency of exceedance over ambient aquatic life water quality criteria was affected by pesticide use, precipitation and irrigation demand in certain periods anteceding the water sampling events.

236 Groundwater Monitoring for regulatory purposes – First steps of a SETAC group to develop a European guidance Document

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The aim of the SETAC Environmental Monitoring Advisory Group on groundwater (EMAG-GW) is to develop a guidance document on the use of groundwater monitoring data for regulatory purposes in the EU. Preliminary feedback on the following topics will be presented: Utilizing monitoring data in regulatory context: Monitoring data can be used in the regulation of pesticides, but use of such data is not straight forward. Groundwater Monitoring "good practices": The design of a monitoring study and the quality of the data are key to generate valuable monitoring results. The study design should be tailored to the questions that the study is expected to answer. Interpretation of GW monitoring data and how representative is the GW monitoring conducted: How to interpret and use monitoring data in regulation and how to assess the representative nature of monitoring are probably the two main recurrent questions associated with groundwater monitoring. These two questions are raised for both compound specific monitoring studies and for public monitoring programmes which may be available. Recommendations are needed on how results should be used and to define what the monitoring data should represent/cover for both types of monitoring studies/programmes. How can these two types of monitoring complement each other?

237 Pesticide Residues in Yamuna, Hindon and Ghaggar Rivers flowing through Vegetables, Rice and Cotton Cropping Areas

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In India cotton and paddy are the major consumers of crop protection chemicals accounting for 50% and 18% respectively. While vegetables consume about 9% share of the crop protection chemicals. As crops are water intensive, it's the quality of the water that is mostly affected by their cultivation, by runoff chemicals in surface water. Therefore a study was undertaken to analyze the contamination by pesticide in surface water samples in flowing near the farms of vegetable, rice and cotton growing areas along with survey for pesticide use by farmers. Yamuna, Hindon and Ghaggar River streams flowing nearby vegetables, rice and cotton crop growing areas. Samples of rivers (Yamuna, Hindon and Ghaggar) were taken from nearby field crop surface, 2km, 2km bottom and 5km. According to survey pesticide sprayed in vegetables, rice and cotton were Cypermethrin and Malathion, Cypermethrin and Endosulfan, Profenofos and Endosulfan, Ethion and Cypermethrin, Endosulfan, Chlorpyrifos Profenofos and Cypermethrin and Cypermethrin respectively. Yamuna River flowing nearby seasonal vegetable growing area, 45% samples were found contaminated with organochlorine and 25% samples with organophosphorous pesticides above MRL value. All the synthetic pyrethroids analyzed were found below MRL value. Hindon River flowing nearby rice growing area and the variety grown there was 11/21. Samples were found contaminated with 52% by organochlorine pesticides, 25% by organophosphorous pesticides and 12.5% by synthetic pyrethroids above MRL value. Ghaggar River flowing nearby desi cotton growing area and the variety grown there were RG-8, HD-123, 38.6% of surface water samples were found contaminated with organochlorine pesticide, 50% by organophosphorous pesticide and 10.5% by synthetic pyrethroids residues which were above MRL value. The results are alarming and show that either the use of banned pesticides (DDT and Endosulfan) is still continued or they are present in surface water samples as they are very persistent. There is need of proper extension services to educate farmers about the judicious use of new molecules of pesticides along with Integrated Pest Management approach to avoid contamination of water resources.

238 Pesticide residues and degradation in vegetative filter strips for long-term environmental exposure assessments

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Understanding and being able to simulate the fate and transport of pesticides from the application on a field, through a vegetative filter strip (VFS), and finally to adjacent receiving water bodies is critical for conducting environmental risk assessments. Previous research has proposed a modeling approach that links the U.S. Environmental Protection Agency's (EPA's) PRZM/EXAMS higher-tier environmental exposure framework with a well-tested process-based model for VFS (VFSMOD), but it assumed that pesticide mass stored in the VFS was not available for transport in subsequent storm events. The objective of this research was to update the modeling approach to consider four formulations for degradation to accommodate different regulatory environments, and then to determine if residues in the VFS and/or estimated environmental concentration (EECs) differed relative to the degradation formulation. The formulations variably consider temperature and moisture content adjustments as proposed by the European Union and USEPA. Results from three distinct 30-yr USEPA scenarios showed that while the type of degradation equation was important in long-term assessments relative to predicting residues at the beginning of each storm event, the degradation formulation was not important relative to EECs.

Everglades and Wetlands Science Part 2: Cellular, Genetic and Toxicological Outcomes of a Changing Habitat

239 Infectious disease linked to climate and genetic variation in amphibian populations of the southeastern United States

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Infectious disease is a well-known driver of animal declines worldwide. North American amphibians have been impacted by to two major emerging pathogens, the fungus Batrachochytrium dendrobatitis (Bd) and iridoviruses in the genus Ranavirus (Rv). Amphibian populations have responded differently to these disease stressors; some populations have declined precipitously while others persist despite ubiquitous infection.

Environmental factors and host genetics may play important roles in disease dynamics, but few studies incorporate both of these components into their analyses. Our study investigates the effect of environmental and genetic factors on Bd and Rv infection prevalence and severity in a biodiversity hot spot, the southeastern United States. We conducted a retrospective infection study on three amphibian species, using population genetics and quantitativepathogen detection to understand how emerging infectious pathogens have impacted natural populations. By combining genetic factors and environmental variables into a general linear model for species with pathogen infection, we elucidate the relative roles of host genetics and environmental variables on predicting disease impact and spread. We conclude that by incorporating both genetic and environmental factors into conservation plans for amphibians, more effective management strategies can be developed to help protect the Southeast's amphibian biodiversity.

240 Genetic and epigenetic variation in Spartina alterniflora following the Deepwater Horizon oil spill

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Catastrophic events offer a unique opportunity to study rapid population response to stress in natural settings. Following the Deepwater Horizon oil spill, we simultaneously examined the genetic and epigenetic structure of recovering populations of Spartina alterniflora, a keystone salt marsh grass. In concert with genetic variation, epigenetic mechanisms like DNA methylation may explain the high resilience ofS. alterniflora to oil exposure, and may offer a rapid mechanism of response to severe environmental challenges, like the Deepwater Horizon oil spill. In this study, we quantified genetic and DNA methylation variation using AFLP and MS-AFLP to test the hypothesis that response to oil exposure in S. alterniflora resulted in genetically and epigenetically based population differentiation. We found high genetic and epigenetic variation within and among sites, and found significant genetic differentiation between contaminated and uncontaminated sites. In contrast, we found the majority of the epigenetic variation occurred within sites and found no significant genome-wide epigenetic differentiation in response to oil contamination. However, despite the lack of genome wide patterns in DNA methylation, we found three MS-AFLP loci (14% of polymorphic MS-AFLP loci) that were correlated with oil exposure. Overall, our findings support genetically based response to selection from the oil spill in this system, but also a limited role for epigenetic mechanisms in population differentiation.

241 Using an in vitro organ culture system to assess the estrogenic effects of CWAF exposure on sex determination in the American alligator

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Coastal aquatic ecosystems are often subject to high levels of anthropogenic activity and contamination. Specifically, these areas are increasingly vulnerable to the threat of oil contamination from offshore oil spills or pipeline ruptures. While a growing body of knowledge exists regarding the toxicity of oil, dispersants, and their mixtures, the potential sublethal biological impacts of dispersants and chemically-dispersed oil still merit further investigation. The American alligator, Alligator mississippiensis, is an ideal aquatic sentinel species due to its regional fidelity, long life as a top predator (bioaccumulation), and its environmental sex determination, in which the temperature and/or estrogenic signals of the developing embryo determines its sex. Exposure to estrogen and certain estrogenic compounds in ovo can result in ovarian development at any temperature in the alligator. This study evaluates the effects of CWAF, the chemical enhanced water-accommodated fraction of crude oil with Corexit, on sex determination and differentiation in the alligator by examining patterns of key mRNA abundances involved in the sex determination process including aromatase, anti-Müllerian hormone(AMH), sex determining region Y-box 9(SOX9) and Forkhead box protein L2 (FOXL2). We revealed that CWAF induced estrogenic activity at nearly 50% of the maximum induction of the endogenous ligand 17β-estradiol (E_2) in transactivation assays with alligator estrogen receptors in vitro. To determine the effects of CWAF at the organ level, gonad-adrenal-mesonephric (GAM) organ complexes were isolated from A. mississippiensis embryos one day prior to the beginning of the thermosensitive period for sex determination and cultured at a male-producing temperature for 1-2 weeks with exposure to media controls, CWAF, and three different concentrations of E_2. After two weeks of GAM culture, E_2 and CWAF exposure induced a female-like pattern in several of the genes examined. The results of this study help to establish a framework for assessing targeted effects of contaminants at the receptor and organ levels, and provide important insight into the potential adverse effects of oil and dispersant exposure on reproductive health in aquatic wildlife as well as in humans.

242 Assessing Estrogenic Activity of the Dispersant Corexit 9500 in Two Aquatic Reptiles, the American Alligator and Diamondback Terrapin

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During the Deepwater Horizon oil spill, millions of gallons of Corexit 9500 were applied to the surface water and at the wellhead to disperse the oil. This massive release mandates evaluation of the long-term effects of Corexit with a focus on endocrine disruption. Estrogen signals play critical roles in temperature-dependent sex determination (TSD) and gonadal differentiation in some turtles and all crocodilians, in which egg-incubation temperatures determine sex of the embryos during a thermosensitive period (TSP). A single exposure to exogenous estrogen during TSP leads to skewed sex ratios by inducing ovarian development, even at a male-producing temperature. Due to this sensitivity to estrogen, reptiles that exhibit TSD, including the American alligator (Alligator mississippiensis) and diamondback terrapin (Malaclemys terrapin), can be sentinel species to investigate chronic exposure to estrogenic environmental contaminants. Both estrogen receptor isoforms, ESR1 and ESR2, were cloned from the diamondback terrapin and found to be highly homologous to painted turtle, alligator, and chicken ESRs. The cloned ESRs were characterized using nuclear hormone receptor transactivation assays in vitro with 17β-estradiol and Corexit. Additionally, alligator eggs were exposed to Corexit at 0.25, 2.5, and 25 μg/g egg weight prior to TSP to investigate the potential endocrine disruption and estrogenic effects on gonadal development in ovo. Gonadal tissues dissected from alligators at one week old were analyzed using histological methods and quantitative PCR to determine resulting sex ratios and understand the outcome of developmental exposure. While Corext showed potential estrogenic activity in vitro using transactivation assays, no significant effects on sex ratios or testicular mRNA abundance were observed as a result of Corexit exposure, suggesting that Corexit alone does not have strong estrogenic activity in ovo. Further investigations are required to assist in understanding the effects of Corexit exposure on the reproductive health of coastal aquatic reptiles.

243 Exploring the epigenetic and biochemical effects of mercury exposure in Diamondback Terrapins (Malaclemys terrapin)

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Mercury is a toxic global pollutant impacting a wide variety of ecosystems across the planet, due to the main route of transmission being atmospheric deposition. Mercury is a potent neurotoxin known to elicit behavioral changes and has been correlated to a myriad of adverse health effects, including congenital heart defects, and reproductive impairment. Sub-lethal, physiological effects of mercury exposure have been difficult to characterize. The Diamondback Terrapin (Malaclemys terrapin) is a long lived, ecologically important mid- to upper trophic level predator in coastal habitats of the Atlantic Ocean, and along the US Gulf Coast. The natural range places this reptilian sentinel in areas historically affected by anthropogenically produced environmental contaminants, including mercury. These qualities make the terrapin an excellent model species for studies of chronic exposure to mercury, as well as a sentinel species for human exposure. In this study, we investigate physiological and epigenetic changes associated with two doses of chronic mercury exposure over a two year time period on an ecologically important reptilian sentinel. Blood samples were analyzed for total mercury content and subsequently analyzed by LC-MS/MS for global DNA methylation patterns and by ^{1}H NMR for metabolomic effects. DNA methylation is a well-studied epigenetic modification that controls gene expression. When DNA methylation patterns are changed as a result of external stimulus, altered gene expression and dysregulation of certain cellular processes can occur. Evaluating DNA methylation changes related to mercury exposure can provide insight into the sub-lethal effects of chronic mercury exposure. Plasma metabolomic profiles were analyzed by multivariate and univariate techniques across doses, gender, and annual time scales. The metabolomics data details the small molecule changes that are taking place as a result of mercury exposure. The combination of these two techniques allows for further elucidation of organism-environment interactions.

244 A means to adapt, a means to disrupt: epigenome-environment dynamics underlying sex determination and reproductive perturbations in the alligator

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Unlike traditional models for reproductive health, the American alligator is a long-lived apex predator that undergoes temperature-dependent sex determination (TSD). Further, these animals are oviparous and field collected eggs allow for the investigation of environmental effects on developmental processes. Here, we use the alligator as a model to (1) examine the role of DNA methylation patterning during TSD and (2) investigate how exposures to endocrine-disrupting contaminants (EDCs) during this period may alter the epigenetic landscape in a manner that influences subsequent reproductive function. When compared to their counterparts living in relatively pristine environments, alligators undergoing natural exposures to EDCs display a severely abated ovarian transcriptional response to gonadotropin stimulation. Here, we employ reduced-representation bisulfite sequencing to explore the sexually dimorphic DNA methylome in gonads from embryos exposed to either male- or female-promoting temperatures. We identify numerous temperature-dependent methylated regions within the alligator genome. In addition, we use targeted bisulfite sequencing on the Illumina platform to examine how DNA methylation status of the CYP19A1 promoter, a gene displaying sexually dimorphic expression and DNA methylation patterns, varies in field-collected embryos originating from contaminated environments. We find that the robustness of sexually dimorphic CYP19A1 promoter methylation is reduced in embryos originating from a site contaminated with EDCs. We next probe the consequences of developmentally inappropriate Estrogen Receptor activity on subsequent CYP19A1 expression and find that treatment with a selective ESR1 agonist prior to gonadal differentiation results in down regulation of CYP19A1 in stage 27 ovaries. Results presented here suggest that DNA methylation patterning may play an integral role in mediating the effects of incubation temperature on sex determination and that EDCs may exert their effects by compromising sexually dimorphic epigenetic patterns acquired during development.

A SETAC Pellston Workshop® on Environmental Hazard and Risk Assessment Approaches for Endocrine-Active Substances Data

245 SETAC Pellston WorkshopTM: Environmental hazard and risk assessment approaches for endocrine-active chemicals (EHRA)

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Suspected endocrine disrupting substances (EDS) are now being evaluated by several regulatory authorities. A debate is in progress about whether or not EDS can be adequately assessed by following the standard approach involving identification of intrinsic hazards, prediction of exposure and consequent calculation of risk or if hazard alone should be used to decide if chemical registration can be permitted. For this reason the SETAC-Pellston Workshop on Environmental Hazard and Risk Assessment Approaches for Endocrine Active Chemicals (EHRA) was held from the 31st of January to the 5th of Febuary, 2016. It had the following aims: 1) resolving the scientific disagreement about how best to assess the impact of endocrine active substances (EAS); developing guidance to assist regulators and policy makers in their decision making and thereby contribute to speeding up the global progress in controlling these chemicals; 3)identifying areas of uncertainty and knowledge gaps. The approach to the workshop was to identify cross-cutting issues that arose from the six case-studies that were performed ahead of the workshop. Cross-cutting issues emerging from the case-studies covered: improved hazard and risk assessment (Coady et al, in review; Marty et al, in review); whether an EDS can confidently be subjected to environmental risk assessment or whether regulation by hazard is the most appropriate option (Parrott et al, in review); distinguishing between endocrine- versus non-endocrine-specific responses (Mihaich et al, in review). Key questions to be addressed to support endocrine disrupter environmental hazard and risk assessment include: environmental exposure; effects on relevant taxa and life-stages; delayed/multigenerational effects; dose-and concentration-reponse relationships. A proposed decision making scheme developed at the workshop will be presented and discussed. Main conclusions of the workshop will be presented as well as areas of future research.

246 Challenges in Assigning Endocrine Specific Modes of Action: Recommendations for Researchers and Regulators

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As regulatory programs focus on evaluating substances for their endocrine disrupting properties, there is a need for careful study design and data interpretation to distinguish between endocrine versus non-endocrine specific responses. This is particularly important where specific criteria are under development to identify endocrine disrupting properties to enable hazard-based regulation. Irrespective of the regulatory process, most jurisdictions use the WHO IPCS definition of an endocrine disruptor (ED), requiring that a substance is demonstrated to cause a change in endocrine function that consequently leads to an adverse effect in an intact organism. Such a definition is broad, and at its most cautious, might capture many

mechanisms that in general would not specifically be considered ED. For instance, stress is a non-specific, neuro-endocrine response that can lead to adverse outcomes. In addition, non-endocrine toxic mechanisms (e.g. hepatotoxicity, acetylcholinesterase inhibition) may operate secondarily or in parallel to impact the endocrine system and apical endpoints downstream. Furthermore, endocrine responses may be adaptive in nature and designed to maintain homeostasis rather than inducing an irreversible adverse effect. The likelihood of indirect effects is increased in (eco)toxicological studies requiring the use of maximum tolerated dose levels, which must produce some adverse effect. The misidentification of indirect effects as truly ED has serious consequences in terms of triggering animal and resource intensive testing and potentially severe regulatory consequences. A review, based on 6 case study substances, was conducted to evaluate scenarios that could complicate the assessment of whether or not a substance is an endocrine disruptor. In order to achieve this objective, a weight of evidence approach was used to evaluate available data for the case-study substances. In this approach, the weight of evidence was based on biological plausibility, empirical support, and essentiality of key events in adverse outcome pathways. A process is recommended where indicative (endocrine specific and non-endocrine specific) and apical endpoints can be evaluated to investigate whether an endocrine mode of action can be conclusively assigned to the effects observed for a given substance.

247 Uncertainties in biological responses that influence hazard or risk approaches to the regulation of endocrine active substances

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Endocrine Disrupting Substances (EDSs) may have certain biological effects including delayed effects, multigenerational effects, and non-monotonic dose response relationships (NMDRs) that require careful consideration when determining environmental hazards. The case studies evaluated for the SETAC Pellston Workshop™: Environmental Hazard and Risk Assessment Approaches for Endocrine-Active Chemicals and other key examples from the literature are discussed. EDSs can have specific and profound effects when exposure occurs during sensitive windows of the lifecycle (development, reproductive). This creates the potential for delayed effects where the adverse effect becomes manifest when exposure has ceased, possibly in a different lifestage. This underscores the need for testing in appropriate (sensitive) lifestages and full lifecycle designs that capture adverse effects wherever they occur in the lifecycle. Such tests are available in the tool box and should be employed to derive endpoints that can be considered protective of all life stages. Similarly, the potential for effects to be manifest in subsequent generations (multigenerational effects) has also been raised as a potential issue in the derivation of appropriate endpoints for EDSs. However, the evidence for such effects as a general issue is limited. Indeed this is reflected in the design of new higher tier tests to assess endocrine active substances (EASs) developed by the OECD and US-EPA that move to extended one-generation designs and away from multi-generational studies for fish and mammals. The occurrence of non-monotonic dose or concentration response relationships is also considered a limiting factor for reliable risk assessment of EDSs. Substantial data reviews are underway to inform on their occurrence. However, evidence to date indicates they are more prevalent in in vitro and mechanistic data, not often translating to adverse apical endpoints that would be employed in risk assessment. A proposal of how to evaluate NMDRs in the context of endocrine hazard and risk assessment procedures is presented. If careful consideration of delayed, multigenerational and NMDR effects are made, it is feasible to assess environmental endocrine hazards and derive robust apical endpoints for risk assessment procedures ensuring a high level of environmental protection.

248 Improving environmental assessment of endocrine active substances

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There has been substantial scientific advancement in the development and implementation of testing, hazard and risk assessment approaches to evaluate potential adverse effects though an endocrine mechanism. In particular, existing test systems and frameworks that have been developed for assessing potential interaction with the estrogen, androgen, and thyroid pathways are relatively comprehensive for identifying and assessing endocrine effects. However, there are now opportunities to retrospectively examine the lessons learned from the recent implementation of these efforts to improve the reliability and relevance of endocrine assessments. Priority areas for improving endocrine screening and testing include: Leveraging information to the extent possible from high through-put screening assays to prioritize and inform testing programs, Using experience with existing assays as a basis for modifying approaches to optimize resource use, Developing additional approaches to address species sensitivity, sensitive life-stages, and critical endpoints to improve the predictive ability to detect an adverse effect at the population level, and Identifying gaps that can be addressed by research to improve testing paradigms. Example ToxCast data sets of endocrine active chemistries will be examined to indicate the utility of high through-put screening to prioritize and inform future testing needs. The example of fathead minnow plasma vitellogenin concentrations across multiple Tier 1 Fish Short Term Reproduction assays will be examined in a post-hoc power analysis to guide future methods development. A summary of the various small fish species used in endocrine screening and testing will be presented to guide species selection based on preliminary chemical information. Finally, research gaps, such as invertebrate endocrine system knowledge, additional endocrine pathways other than estrogen, androgen, and thyroid pathways, and other needed research in the area of endocrine activity will be suggested. Collectively, these recommendations can reduce uncertainty in testing and assessment approaches that evaluate potential adverse effects exerted though an endocrine mechanism.

249 Population-Relevant Endpoints in the Evaluation of Endocrine-active Substances (EAS) for Ecotoxicological Hazard and Risk Assessment

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For ecological risk assessment, endocrine disrupters require the establishment of an endocrine mode of action (MoA) with a plausible linkage to a population-relevant adverse effect. Current ecotoxicolgy test methods mostly incorporate apical endpoints, although, some also include mechanistic endpoints, at the subcellular through organ level, which can help establish an endocrine MoA. However, the link between these endpoints and adverse population-level effects is often unclear. Case studies of endocrine-active substances (EAS) (tributyltin, ethinyl estradiol, perchlorate, trenbolone, propiconazole, and vinclozolin) were used to evaluate the population relevance of toxicity endpoints in various taxa according to the OECD Conceptual Framework for Testing and Assessment of Endocrine Disrupters. For some taxa, such as mollusks, the population relevance of tributyltin-induced gastropod imposex is well established. However, for other taxa, the population relevance of observed effects is not as well understood. Furthermore, potential adaptation and recovery processess also are important to consider when evaluating the adverse effects of EAS exposure on wildlife populations. As our understanding of endocrine perturbations and key event relationships improves, adverse population-level effects will be more easily and accurately predicted. This presentation will focus on examples of potential population relevance from these case study chemicals as well as briefly discuss future needs to better predict population-level effects resulting from EAS exposure.

250 Evaluating the Credibility of Histopathology Data in Environmental Endocrine Toxicity Studies

J.C. Wolf, Experimental Pathology Labs, Inc.; G. Maack, Federal Environment Agency / Ecotoxicological Assessment

Agencies responsible for environmental protection are tasked with developing regulatory guidance that is based on the best available scientific evidence. Histopathology is a common endpoint in toxicologic bioassays; however, because of the subjective nature of this endpoint, and the advanced level of specialized training required for its effective utilization, the reliability of histopathology data can be inconsistent. For the present study, a total of 189 papers that involved investigations of endocrine active substances (EAS) were reviewed for the credibility of their reported histopathology findings. The review process incorporated standardized criteria for article and data selection, and systematic procedures for data evaluation and histopathology credibility (HC) scoring. A major outcome of these efforts was the finding that 54% of the examined papers contained histopathology data that were considered to be either highly credible or credible, whereas such data were deemed to be of equivocal, dubious, or no credibility in 46% of cases. The ultimate goals of this work are to draw attention to reliability issues that can affect the histopathology endpoint, provide recommendations to improve the quality of this endpoint, and suggest an approach for the expeditious and judicious use of histopathology data in weight of evidence determinations required for hazard and/or risk assessment. This exercise was conducted initially as part of a SETAC-Pellston Workshop™ entitled "Environmental Hazard and Risk Assessment Approaches for Endocrine-Active Chemicals (EHRA): Developing Technical Guidance Based on Case Studies to Support Decision Making" that was held in Pensacola, Florida, USA, from January 31st to December 5th, 2016.

Multi-ion Toxicity: Mechanisms and Effects

252 Thoughts on applying existing toxicological understanding to risk assessment for major ions in fresh waters

D.R. Mount, R.J. Erickson, B. Forsman, T.L. Highland, R. Hockett, D.J. Hoff, C.T. Jenson, T.J. Norberg-King, USEPA / NHEERL / Mid-Continent Ecology Division; D.J. Soucek, Illinois Natural History Survey / Illinois Natural History Survey

Recent research in our laboratories and many others have greatly increased understanding of the responses of freshwater organisms to increased concentrations of major geochemical ions (Na, K, Ca, Mg, Cl, SO4, HCO3) in laboratory toxicity tests, stream mesocosms, and in natural streams. The lessons learned to date raise challenges to developing assessment approaches that effectively integrate knowledge from studies of all types. For example, studies of benthic community data from the field suggest that the conductivity associated with changes in those communities varies with the natural background in the study area, but it is not as clear how laboratory toxicity data should be viewed or applied with respect to varying background. Most laboratory toxicity studies involving major ions are conducted using single salts (e.g., NaCl, $MgSO_4$), but most exposures in natural waters involve elevation of multiple ions to varying degrees. As a specific example, how do we interpret toxicity data for $MgSO_4$ alone at low Ca, when in the field elevated Mg usually co-occurs with at least some elevation of Ca as well? And further, how do we balance the practical appeal of guidelines based on single ions (e.g., Cl or SO_4) with our understanding that toxicity is a function of the complete ionic composition of the water?

253 Can major ion uptake kinetics and interactions inform mechanisms of multi-ion toxicity in aquatic insects?

S.A. Scheibener, D. Buchwalter, North Carolina State Univ / Dept of Biological Sciences

Despite a recent surge in interest in freshwater salinization and its consequences to biodiversity, both the scientific and regulatory communities remain relatively uninformed about the mechanisms of multi-ion toxicity. Opinion remains divided as to whether toxicity results from particular individual ionic concentrations, ionic ratios or total salinity/conductivity. Even in the context of evaluating the toxicity of simple salts (e.g. sodium chloride or sodium sulfate), we typically assume toxicity is the result of the anionic concentration, but we don't know that this is 100% accurate. In the face of so many uncertainties, it is important to begin building a fundamental physiological foundation for understanding multi-ion toxicity. In this talk, we will present the results of ongoing studies that examine flux rates of individual ions as a function of their concentration and the interactive effects of other ions in aquatic insects. We show that extreme increases in Mg concentrations are required to out-compete Ca transport. We provide some evidence for an antagonistic effect of potassium, calcium and sulfate on sodium transport. We also compare the kinetics of Na (as NaCl) and SO_4 (as Na_2SO_4) in Neocloeon triangulifer. For both ions, transport rates continue to increase at concentrations beyond those found in the most contaminated freshwaters, suggesting that this species lacks regulatory capacity for controlling uptake rates as concentrations increase. In general, transport rates of these two ions at equimolar concentrations are 2-5 fold high for Na than for SO_4,though both have not been studied under identical ranges. We hope to also present the results of ongoing dual-labeled isotope (^{22}Na and $^{35}SO_4$) experiments that assess the relationship between Na and SO_4 uptake rates under ionic ratio variations.

254 The Mechanistic Toxicity of Multi-Ion Exposures in Pimephales promelas (Fathead Minnow)

K.A. Johnson, Clemson Univ / Inst of Environmental Toxicology; P. van den Hurk, Clemson Univ / Biological Sciences; S.J. Klaine, Clemson Univ / Inst of Environmental Toxicology

Dissolved ions have many important physiological functions within freshwater organisms. Particularly, these ions are necessary for the formation of electrochemical gradients within cells. These gradients produce a delicate balance that controls water homeostasis and further ion movement within the organism. Because the internal ion concentration of freshwater organisms is typically greater than their external environment, it is necessary for them to utilize active transport through a series of pumps and transporters in order to maintain this homeostatic balance. However, due to agricultural irrigation, mining activities, road-salt runoff, and other anthropogenic sources, dissolved ion concentrations in the aquatic environment can become significantly elevated. An increase in dissolved ion levels ultimately leads to an increase in salinity. If the external ion concentration exceeds what is physiologically tolerable for freshwater organisms, then the ionoregulatory function or acid-base balancing capabilities of different organs may become dysfunctional. Seven day static-renewal exposures were conducted with chloride, sulfate, and bicarbonate as variable single ions and in binary mixtures to investigate the underlying mechanisms that lead to ionoregulatory impairment. These mechanisms were evaluated by measuring carbonic anhydrase activity, Na^+/K^+-ATPase activity, and major ion concentrations within individual organs important for ionoregulation, specifically the gill, kidney and intestines. The results of this work provide further insight into the mechanisms of toxicity of ion mixtures to freshwater organisms and serve as a foundation for the development of predictive models to manage water quality.

255 Acute and Chronic Ceriodaphnia Toxicity As a Result of Ion Imbalance and Magnesium Based Hardness

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An effluent from a food and beverage manufacturer caused chronic lethal and sub-lethal toxicity and occasional acute toxicity to Ceriodaphnia dubia. Using recent literature, multiple lines of evidence established that ion imbalance was the cause of observed toxicity. Due to various cleaning procedures in the manufacturing process and the use of magnesium hydroxide in the treatment process, effluent hardness was in excess of 1500 mg/L and a 10:1 ratio of magnesium to calcium was often observed. Correlation analyses implicated increased use of magnesium hydroxide as the cause of toxicity. Using a simulated effluent with various ion characteristics observed in the actual effluent, it was concluded that magnesium was a primary cause of C. dubia acute and chronic toxicity. When non-toxic effluent samples were treated with magnesium hydroxide at concentrations similar to those observed in toxic samples, toxicity effects were observed in the effluent. Reducing the ratio of magnesium to calcium reduced or eliminated effluent toxicity.

256 Comparing responses and dose metrics for mayflies and daphnids exposed to major ion salts

D.J. Soucek, Illinois Natural History Survey; A. Dickinson, Illinois Natural History Survey / Ecology; D.R. Mount, R.J. Erickson, R. Hockett, USEPA / ORD NHEERL Mid-Continent Ecology Division

Field studies have shown that mayflies (Ephemeroptera) tend to be more sensitive than other benthic macroinvertebrates to elevated levels of total dissolved solids in streams. While work with other species has shown that major ion toxicity is dependent on the ionic composition of the water, little is known about how ionic composition influences responses of mayflies to elevated major ions. We tested the toxicity of major ion salts to the mayfly Neocloeon triangulifer using dilution waters in which we manipulated either the concentrations of all background ions, Ca:Mg ratios, or sodium and potassium concentrations. We also conducted acute toxicity tests with D-mannitol and sodium gluconate to investigate potential effects of high osmolarity in the absence of "toxic" cations or anions (D-mannitol) or in the absence of a "toxic" anion (sodium gluconate). Finally, we conducted full-life chronic toxicity tests with two sodium salts. In the case of the sodium salts, expressing LC50s (acute tests) or EC20s (chronic tests) in terms of sodium activity (mM) produced a much narrower range of effect levels than when expressing the same data in terms of the anion activity. Furthermore, exposing mayflies to high osmolarity using D-mannitol resulted in a minor effect on survival, whereas the sodium gluconate test resulted in a sodium activity effect level similar to those produced with inorganic sodium salts. This suggests that sodium, rather than overall osmolarity, was responsible for observed toxicity. Comparing general trends in major ion toxicity for Neocloeon to those observed for Ceriodaphnia dubia revealed similarities in some cases but differences in others. For example, while Na activity appears to be the driver of toxicity to the mayfly in sodium salt tests (NaCl, Na_2SO_4, $NaHCO_3$), for C. dubia, the mechanism of acute toxicity associated with Na salts appears to depend on multiple ions and is closely correlated with osmolarity. While the two species are similar in that it appears the acute toxicity of magnesium and potassium salts may be driven by the cation as well, the species are different in terms of their relative responses to Na, K, and Mg. The acute toxicity of potassium to the mayfly is similar to that of sodium on a mM activity basis, and LC50s for Na and K are ~4-fold greater than those for Mg on a mM activity basis, whereas for C. dubia, K and Mg LC50s are similar, and they are ~4 to 6-fold lower than those for Na on a mM activity basis.

257 The acute and chronic toxicity of major geochemical ions to Hyalella azteca – Ion interactions and interspecies comparisons

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We have previously reported that the acute and chronic toxicities of major geochemical ions (Na, K, Ca, Mg, Cl, SO4, HCO3) to Ceriodaphnia dubia can involve multiple, independent mechanisms. The toxicities of K, Mg, and Ca salts were best related to the chemical activity of the cation, while the toxicities of Na salts also reflected an influence of other ions and were well correlated with osmolarity. The toxicities of Na and Mg salts were also inversely correlated to Ca and the toxicity of K salts were similarly inversely correlated to Na. However, research by us and others on other freshwater species, including insects, molluscs, and fish, have shown certain inter-species differences regarding these relationships. In this talk, we will report on major geochemical ion toxicity to another crustacean, Hyalella azteca. This species also shows both similarities to and differences from C. dubia. Notable similarities include a high toxicity of K, independent action of Mg and Na salts, and a strong ameliorative effect of Ca on the toxicities of Na and Mg salts. Notable differences included significant variation in the relative toxicities of different salts, and sodium salt toxicity not being correlated with osmolarity but rather suggesting a more specific role of sodium and sulfate. Unlike C. dubia, H. azteca also shows marked differences in the acute:chronic ratios across different salts. This work further emphasizes the need to consider interspecies differences in developing assessment methods for complex ion mixtures. The contents of this abstractneither constitute nor necessarily reflect USEPA policy.

258 Progress in Predicting the Aquatic Toxicity of Mixtures of the Major Ions Using Mechanistically Based Models

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This exploratory work addresses the aquatic toxicity of mixtures of the seven major ions Ca, Mg, Na, K, Cl, SO_4, and HCO_3. Attempts to develop a water quality criterion for the concentration of any single ion (for example, Cl) and attempts to develop a criterion for the summation of ion concentrations (for example, conductivity or total dissolved solids) are confounded by the influence of the mixture composition on toxicity. Searching for unifying principles for explaining how composition affects toxicity, this work describes further progress in using transepithelial potential (TEP) calculated from Nernst Equation concepts, specifically the Spangler modification of the Goldman, Hodgkin-Katz (GHK) Equation. It also considers whether the Strong Ion Difference approach might have potential for application. Results will be presented for model applications to a few thousand toxicity test treatments from studies of mayfly, daphnids, and fathead minnow. Model strengths and limitations will be described.

259 Predicting the Aquatic Toxicity of Mixtures of the Major Ions: The Need for Speciation

R. Bubnyte, HDR inc. / Water Quality Modeling; W.J. Adams, Rio Tinto; K. Brix, EcoTox / Zoology; C.G. Delos, Great Lakes Environmental Center; K.J. Farley, Manhattan College / Civil and Environmental Engineering; J. Goodrich-Mahoney, EPRI / Environmental Dept; P.R. Paquin, Hydroqual / Water Quality Modeling

The development of water quality criteria for the seven major ions Ca, Mg, Na, K, Cl, SO_4, and HCO_3 has proven to be a challenging endeavor. This is because the toxicity of any individual constituent has been found to be influenced by both the concentrations of the other dissolved solids that are present and by mixture composition as well. One approach that has been used to address multi-ion toxicity (MIT) is to use an integrative measure (e.g., total dissolved solids or conductivity) to quantify the concentration of the overall mixture of major ions present, and to deal with variations in mixture composition on a site-specific basis. Another alternative that has had some success is to use a multi-variate statistical modeling approach. Our efforts have pursued the problem from a different perspective, by computing trans-epithelial potential (TEP) based on the internal and external concentrations of the major ions and model parameters that are evaluated by calibration to toxicity data. The analyses to be presented here will compare results obtained using two alternative approaches that have been used to do this: assuming complete dissociation of the major ions (the simpler approach) versus a more detailed approach that considers chemical speciation. The advantages and disadvantages of these alternative approaches will be discussed. The findings will be illustrated using modeling analyses of MIT toxicity test results for relatively sensitive aquatic invertebrates.

Computational Toxicology: Integrating -Omics and Chemistry to Identify Chemicals of Environmental Concern

260 Integrating chemical monitoring data with high-content and high-throughput effects data to prioritize contaminants and hazards in chemical mixtures

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Determining ecological risks associated with exposures to complex chemical mixtures in the environment is one of the main challenges of chemical safety assessment and environmental protection. Effects-based monitoring tools that can measure the integrated biological activity of an entire mixture have been proposed as one of the solutions. An important limitation of the effects-based approaches is that they typically do not provide insight into which chemicals are causing the observed biological responses. Current approaches for integrating chemical monitoring with biological effects data will be discussed. More specifically, we will present and critically evaluate two approaches where prior knowledge regarding toxicity of individual contaminants is combined with empirical in situ assessment to predict toxicity of mixtures and prioritize contaminants. The first approach involves development of knowledge assembly models (KAMs; which is specific to the aquatic system of interest) based on chemical monitoring data and publically available chemical-gene interaction data. Follow-up fish transcriptomics studies and reverse causal reasoning approaches are then utilized to prioritize risks and contaminants. The second approach utilizes publically available high-throughput in vitro data to extract benchmark effect concentrations for hundreds of biological targets. From these data, exposure-to-activity ratios are calculated and integrated with empirical in vitro effects assessment to prioritize specific chemicals for further testing and to identify biological effects of interest. The observed or predicted molecular-level effects data generated by these two approaches can be integrated with adverse outcome pathway knowledge to aid extrapolation into regulatory outcomes of concern (e.g., organism- and population-level effects). The contents of this abstract neither constitute nor necessarily represent official USEPA views and policies.

261 A Bayesian network model for predicting aquatic toxicity mode of action using two dimensional theoretical molecular descriptors

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The mode of toxic action (MoA) has been recognized as a key determinant of chemical toxicity but MoA classification systems in aquatic toxicology have been limited. We developed a Bayesian network model to classify MoA using a recently published dataset containing over one thousand chemicals with aquatic toxicity MoA assignments. Two dimensional theoretical chemical descriptors were generated for each chemical using the Toxicity Estimation Software Tool. The model was developed through augmented Markov blanket discovery from the dataset with the MoA broad classifications as a target node. From cross validation, the overall precision for the model was 80.2% with a R^2 of 0.959. The best precision was for the AChEI MoA (93.5%) where 257 chemicals out of 275 were correctly classified. Model precision was poorest for the reactivity MoA (48.5%) where only 48 out of 99 reactive chemicals were correctly classified. Narcosis represented the largest class within the MoA dataset and had a precision and reliability of 80.0%, reflecting the global precision across all of the MoAs. False negatives for narcosis most often fell into electron transport inhibition, neurotoxicity or reactivity MoAs. False negatives for all other MoAs were most often narcosis. A probabilistic sensitivity analysis was undertaken for each MoA to examine the sensitivity to individual and multiple descriptor findings. The results show that the Markov blanket of a structurally complex dataset can simplify analysis and interpretation by identifying a subset of the key chemical descriptors associated with a MoA and provide a network classification model with reasonable prediction accuracy.

262 Computational model for Nrf2-ARE activation in Human HepG2 cells based on whole-molecule chemical properties and mechanistic domains

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The development of in silico with high sensitivity and specificity is instrumental to the rational design of safer commercial chemicals. Exposure to a variety of commercial chemicals has been associated with reactive oxygen species (ROS) imbalance and oxidative stress (OS), implicated in adverse outcomes such as immune suppression, cancer, neurodegenerative, rheumatoid and cardiovascular diseases. The critical biochemical pathways involved in OS response are largely induced by transcription at antioxidant response element (ARE), which is triggered by covalent modification of the Keap1-Nrf2 complex. Here we present a quantum-mechanical in silico model for ARE induction triggered by adduct formation on the reactive. The model is based on curated in vitro ARE assay data from the EPA's ToxCast/Tox21 initiative, and a data set of > 1000 plasticizers, pesticides, food additives, antimicrobials, and discontinued pharmaceuticals. The likely mechanism for covalent interaction, including acylation, Michael addition, Schiff base formation, and nucleophilic aromatic and aliphatic substitution were identified for each chemical. Classification and Regression Tree (CART) and Support Vector Machine (SVM) models were then developed for each one mechanistic domain using quantum mechanical descriptors and physicochemical properties, calculated taking into account ionization at biological pH. We find that the effectiveness of global parameters at explaining and predicting ARE response depended on structural domain, with several domains showing train and test accuracy > 80% for classification into inactive, weakly active and strongly active chemicals. The work shows the importance of mechanistic considerations in in silico assessments and highlights the opportunities and limitations of global molecular parameters when estimating in vitro biological responses.

263 Integrating multiple levels of information to support Danube risk assessment in SOLUTIONS

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Environmental monitoring programs, such as the Water Framework Directive in the European Union or the U.S. Clean Water Act (CWA), have accumulated vast amounts of data on contamination and ecology of surface waters within their respective jurisdictions. In parallel, chemical registration processes have resulted in a wealth of chemical property and organism specific single chemical toxicity data. However, it remains a great challenge to link the occurrence of chemical stressors with the observed toxicity in the environment. The SOLUTIONS project was envisaged to address many of these challenges and develop tools and methodologies to support European environmental and water policy decisions [1]. Central to the SOLUTIONS project is the focus on effect based risk assessment using a wide selection of bioassays both in vitro and in-vivo to establish the effects of single chemicals and complex mixtures [2]. A component of this large project is to establish the usability of whole organism transcriptomics data to support bioassay results, hotspot, and emerging pollutant identification. In this presentation we will show the details of the SOLUTIONS project and the application of OMICs to understand the molecular responses of Daphnia magna to chemical stressors in the Danube River Basin. By integrating the various data available in the SOLUTIONS consortium we are able to identify molecular responses directly linking from compound to in vitro bioassay and to broader D. magna molecular responses in an Adverse Outcome Pathway like paradigm. We show that utilizing available toxicological knowledge we can identify some linkages, but that much of the observed response remains unexplained. We show that by approaching this challenge with an unbiased computational methodology we can identify stressors for which little or no toxicological knowledge is available.

264 Computational Predictive Toxicology From Molecular to Geospatial Scales: A Case Study of Inorganic Arsenic

L. Burgoon, US Army Engineer Research and Development Center / Environmental Laboratory

Environmental contamination of drinking water sources with inorganic arsenic may lead to increased health impacts, including cancer, diabetes, and cardiovascular disease. We've used the AOPXplorer as a predictive toxicology tool to enable us to predict potential adverse outcomes, and molecular modes of action for diseases, using transcriptomic and literature information. By coupling these predictions with data from genome-wide association studies (GWAS), single nucleotide polymorphism and population occurrence information, as well as population data from the US Census, we can begin to identify potentially susceptible populations. By combining this information together with water quality information, we are taking a geospatial approach to make predictions about the likelihood of adverse health impacts at the community level. We will also demonstrate how these models help inform decision-making by using a game theory approach to identify the potential impacts of various environmental and policy scenarios.

265 Shifts in microbial community composition and function due to acid mine drainage pollution of Hengshi River (Southeast China)

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Acid mine drainage (AMD) is one of the most environmentally threatening byproducts of the mining industry. Despite its extreme toxicity and acidity, many environments receiving AMD harbor numerous acidophilic and metal-tolerant microorganisms; however, our understanding of the roles of geochemical factors in shaping microbial community structure and the function of microorganisms in natural attenuation of AMD is limited. Located in Southeast China, the Hengshi River is continually contaminated by the Dabaoshan mine, providing an opportunity to explore the effects of AMD-impacted environments on microorganism community composition and function. In this study, twenty-seven sediment samples including five controls were collected from heavily contaminated upstream to lightly polluted downstream sites. Geochemical, and next-generation and high-throughput sequencing analyses were integrated to characterize the spatial distribution and function of microbial communities along the pollution gradient. Geochemical/physicochemical parameters (e.g., pH, conductivity, total organic carbon) of water and sediments significantly differed among samples collected from environments that differed in their extent of contamination with heavy metals (Cd, Cu, Zn, Pb, and As). 16S rRNA sequencing results revealed that bacterial richness and evenness gradually increased along the river with decreasing contaminant levels, which was supported by beta diversity. Most abundant prokaryotic organisms in all samples belonged to the Firmicutes and Proteobacteria phyla. Other phyla, such as Actinobacteria, Acidobacteria, Bacteroidetes, and Nitrospirae that have previously been reported to be characteristic for AMD contaminated environments were also detected in Hengshi samples. At the genus level, Acidocella, Leptospirillum, Thiomonas, and Acidiphilium were significantly dominant in samples from heavily contaminated upstream, compared to the samples from downstream and reference sites. Furthermore, comparative metagenomic analysis indicated that the microbial communities in upstream sites harbored more genes/enzymes for heavy metal detoxification, sulfur oxidationand tolerating low pH, respectively, suggesting diverse metabolic capacities along the pollution gradient of the Hengshi river. Our findings offer an initial insight into the patterns of microbial communities structure and function along the river system contaminated by AMD.

266 Investigating relationships between contaminant and metabolomics profiles in polar bears (Ursus maritimus) from the Hudson Bay Region of Canada

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Polar bears (Ursus maritimus) are the apex predators of the arctic marine food web, and as such are susceptible to the bioaccumulation and biomagnification of a wide range of persistent organic pollutants (POPs) and contaminants of emerging concern from their food sources. Given the wide range of modes of action of the contaminants, determining the effect of mixtures on wildlife has proven to be a challenge for toxicologists. Using metabolomics profiling, changes in hundreds of endogenous metabolites in the tissues of wildlife can be measured in response to many types of stressors, including contaminant exposure. Laboratory experiments have shown significant changes to metabolomic profiles due to contaminant exposure in fish, but few have applied these techniques to field studies. In the present study, tissues were collected from the Southern (SHB) and Western Hudson Bay (WHB) polar bears (n = 41), for the analysis of contaminants (fat and liver) and endogenous metabolites (liver). Approximately 200 contaminants and 218 metabolites (amino acid, biogenic amines, bile acids, fatty acids, and phospholipids) were used to build profiles in the bears, and comparisons were made between males and females from the SHB, and between male bears from the SHB and WHB. Metaboanalyst^{TM} was used to apply partial least squares discriminant analyses (PLS-DA) in conjunction with variable importance projection (VIP) to identify metabolites and contaminants that had a large influence on the separation of the PLS-DA scores. Oxychlordane was the only contaminant identified by the VIP analysis comparing males and females, while the sum of the hexose sugars, 3 polyunsaturated fatty acids (all n-6 fatty acids), 1 monounsaturated fatty acid, 1 saturated fatty acid, and 1 phosphatidylcholine (PC) were also identified. Oxychlordane and all of the metabolites except docosahexaenoic acid were significantly greater in female bears over males. Between the SHB and WHB males, only perfluoro-4-ethylcyclohexane sulfonic acid (PFEtCHxS), and 4 PCs were identified by the VIP analyses. The SHB bears had significantly greater concentrations of PFEtCHxS, and significantly lower concentrations of all of the PCs than the WHB bears. Interesting to note is that male and female metabolomic differences were related to an organochlorine, while the two male populations were differentiated by a perfluorinated surfactant. The potential implications for the health of the polar bears will be discussed.

267 Gradient of evolved resistance to contaminants in Gulf killifish (Fundulus grandis) populations from Galveston Bay, Texas, USA

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The Houston Ship Channel (HSC) in Texas includes areas that have historically been found to contain substantial levels of mercury, dioxins, furans, polychlorinated biphenyls (PCBs), and polycyclic aromatic hydrocarbons (PAHs). We collected Fundulus grandis from contaminated areas on the HSC, from sites expected to have intermediate contamination, and from reference sites to establish a total of 12 lab colonies. F1 embryos from HSC populations were up to 1,000x more resistant to PCB126- and 2-5x more resistant to coal tar-induced cardiovascular teratogenesis. We confirmed biparental inheritance and a genetic basis of protection through reciprocal crosses between a reference and contaminated population, and by conducting experiments on F2 embryos for select populations. Similar to patterns observed for Fundulus heteroclitus inhabiting polluted areas along the US Atlantic coast, HSC populations of F. grandis exhibit a reduction of both basal and induced cytochrome P450 1A (CYP1A) activity, suggesting a common mechanism of adaptation. We observe a gradient of adaptation that correlates with the level of pollution at multiple HSC sites. Thus, we sequenced 288 individuals from 7 populations to understand the genetic causes for this resistance. In addition, we look at contaminants in various environmental matrices to be able to understand the chemical drivers of the selective sweeps that we see in F. grandis populations. Such results suggest that the HSC can serve as a “natural laboratory” to study evolutionary processes driven by anthropogenic pollution.

Fate and Effects of Metals: Mechanisms of Toxicity

268 Role of biologically mediated boundary reactions in the bioavailability of cadmium to freshwater phytoplankton

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Recent studies have suggested that low-molecular-weight (LMW) ligands can enhance metal uptake by marine phytoplankton. We have extended this work to freshwater phytoplankton and have investigated Cd bioavailability in the presence of LMW thiols, with two green algae (Chlamydomonas reinhardtii two strains CPCC11 and CC1690, and Pseudokirchneriella subcapitata) and a cyanobacterium (Anabaena flos-aquae) as our model species. Using nitrilotriacetic acid (NTA) as a metal buffer to hold the free Cd^{2+} concentration constant, we progressively added cysteine to the exposure medium and determined short-term Cd uptake rates; the Cd uptake rates remained constant and were unaffected even when the concentrations of the cysteine-Cd complexes exceeded that of the free Cd^{2+}. Similar results were obtained with green algae grown with different inorganic nitrogen sources (to affect their ability to utilize amino acids) and low zinc concentrations (to affect their ability to take up Cd), and also for other LMW thiols, including penicillamine, mercapto-succinic acid, dithiothreitol and glutathione. These results suggest that the thiol-Cd complexes are not taken up by the algae and that the formation of hypothetical ternary surface complexes with the Cd transporter molecules ‘X’, e.g. cysteine-Cd-X-alga, does not contribute to overall Cd uptake. However, when the experiments were repeated with a fixed free Cd^{2+} concentration, either buffered with NTA or simply complexed with cysteine in the absence of NTA, a significantly higher Cd uptake rate was observed in the presence of cysteine than in the control cultures (where $[Cd^{2+}]$ was buffered by NTA alone). We speculate that the increased Cd bioavailability was due to an enhancement of the free Cd^{2+} concentration in the boundary layer, resulting from chemical oxidation of metal-binding ligands by oxidants released by the algae. Meanwhile, only in the nitrate-acclimated CC1690, a significantly lower Cd uptake rate was observed in the presence of cysteine than in its control (buffered by NTA). This decreased Cd bioavailability may result from a decrease in the free Cd^{2+} concentration in the boundary layer, resulting from de-protonation of metal-binding ligands induced by local pH enhancement as a consequence of algal release of HO^-. The results highlight the importance of biologically-mediated surface processes, in addition to other well-known abiotic processes, in determining metal bioavailability.

269 Toxicity of metal-contaminated sediments from the Upper Columbia River, Washington, to early-life-stage White Sturgeon (Acipenser transmontanus)

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The Upper Columbia River (UCR) has been contaminated with metals from effluents resulting from smelter and mining activities. Approximately 10 million metric tons of slag, in addition to liquid effluent, were released from a lead-zinc smelter in Trail, British Columbia from 1947 until 1995, resulting in some UCR sediment metal concentrations being elevated above biological criteria concentrations. In the transboundary reach of the UCR, White Sturgeon population declines have been associated with little to no natural recruitment since the late 1960s. Copper, which is present in significant concentrations in slag, can be mobilized from slag containing sediments and is highly toxic to early life-stage (ELS,1.0 for three of the six sediment treatments tested. Chronic toxic units for copper estimated with a biotic ligand model were also >1.0 in three of the six sediment treatments with both measures of toxic units >1.0 in two of those three treatments. Toxic units >1.0 suggest toxicity may occur. Sturgeon exposed to sediment collected near the city of Northport, WA, which had toxic units >1.0 for both measures, had significant increases in mortality and significant decreases in swimming activity, compared to sturgeon in the control treatment, which were exposed to clean quartz sand. No significant effects were observed in the other sediment treatments. This study sheds light on the relationships between exposure to metals associated with slag and adverse effects on the White Sturgeon population.

270 Subcellular metal partitioning in white suckers exposed to metal-mining effluents: a relevant tool to improve risk assessment for aquatic organisms

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Discharges from metal mining operations may lead to metal accumulation and toxicity in exposed aquatic species. Indeed, once metals enter cells, they can bind to sensitive components and cause deleterious effects. Nevertheless, metals can also be detoxified by binding to molecules designed to sequester them and limiting their toxic effects. The objective of this study was to assess the subcellular distribution of metals in white suckers (Catostomus commersoni) exposed to metal-mining effluents, and to relate metal accumulation in specific fractions to toxicity. Mature male and female fish were collected downstream from a metal-mining effluent and in a reference area, and metal partitioning (As, Cd, Cu, Ni, Pb, Se and Zn) among potentially metal-sensitive fractions and detoxified metal fractions in livers and gonads was determined after differential centrifugation and heat-denaturation steps. The liver somatic index (LSI) was significantly lower in exposed fish than in reference fish for males and females, and the gonadosomatic index (GSI) was significantly lower in exposed females. Total hepatic metal concentrations were significantly higher in exposed fish than in reference fish, with Cd, Cu and Se being accumulated the most; no differences between sexes were observed.

However, marked differences in Cu, Se and Zn concentrations between exposed male and female gonads were observed, all being lower in males. Total metal concentrations in female gonads were significantly higher in exposed fish compared to reference fish for Se, Cd and Cu. Subcellular metal fractionation showed that in livers and gonads, 50% of Cd and Cu burdens were found in the heat-stable cytosolic proteins fraction, which includes metallothioneins. In contrast, the largest contributor to the total Se liver and gonad burdens was the potentially metal-sensitive heat-denaturable proteins fraction (livers, 30%; gonads, 50%), and also the mitochondrial fraction in livers (≈ 15%). These results suggest that Cu and Cd were well detoxified and regulated by white suckers, whereas the presence of relatively high Se concentrations in potentially metal-sensitive fractions suggests that exposed fish were subject to stress, likely partly linked to the observed decrease of LSI and GSI. The advantages of subcellular metal partitioning, as a tool to better identify metals of potential concern for risk assessment, will be discussed.

271 Copper toxicity to Florida apple snail (Pomacea paludosa) and development of a Biotic Ligand Model with P. paludosa

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The long history use of copper (Cu) in South Florida has resulted in Cu contamination in its ecosystem. Pomacea paludosa is a key species in the South Florida ecosystem and plays an important role as a main food source for many higher trophic organisms, including the snail kite. Our previous studies found that P. paludosa is sensitive to Cu and the toxicity of Cu to P. paludosa is influenced by water quality characteristics. These results revealed that P. paludosa is a good species for the Biotic Ligand Model (BLM). The objective of the present study is to calibrate and validate the US Cu-BLM to P. paludosa in support of development of water quality guidelines for Cu and the South Florida ecosystem, including the Everglades. Copper toxicity tests were conducted with P. paludosa in formulated water that had a wide range of water quality parameters and organism age. The median lethal accumulation concentration (LA50) and the Cu-biotic ligand binding constant for P. paludosa were also determined. Results of the present study were used for calibrating the BLM. In general, the BLM works well with P. paludosa. There was a strong correlation between predicted and measured toxicity. An important note is that organism age plays an important role in sensitivity to Cu. The snail BLM developed in the present study can be used for development of species sensitivity distribution in support of setting site specific water quality criteria for Cu in the South Florida ecosystem.

272 Mechanisms of Selenium-Induced Spinal Deformities in Fish

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Selenium toxicity to oviparous vertebrates is often attributed to selenomethionine (SeMet), which can biomagnify and be passed to offspring through maternal transfer. Although oxidative stress is implicated in SeMet toxicity, knowledge gaps remain in mechanisms of SeMet-induced spinal deformities. In the present study, we use the Japanese medaka (Oryzias latipes) model to investigate the role of oxidative stress, cell death, and the unfolded protein response (UPR) on skeletal gene expression and SeMet toxicity, linking localization of cellular effects to observed abnormalities. Medaka embryos were treated with 2.5µM or 5µM SeMet for 24hr at stage 25 (48 hours post fertilization). Post treatment, embryos were separated into normal, deformed (mild, moderate or severe), or dead categories. Dichlorofluorescein staining demonstrated oxidative stress in tails of embryos with observable spinal malformations. Furthermore, acridine orange staining for apoptosis identified significantly more dead cells in tails of treated embryos. Gene expression studies for the UPR suggest a role for CHOP (c/ebp homologous protein) induced apoptosis in dead and deformed embryos after 5µM SeMet, accompanied by a decrease in PDIA4 (protein disulfide isomerase A4) and no change in Dnajb9 (ER DNA J Domain-Containing Protein 4). This expression was distinct from the UPR induced by well-studied ER stress inducer, tunicamycin, which robustly activated CHOP, PDIA4 and Dnajb9. Finally, SeMet treatment significantly decreased transcripts of skeletal development, Collagen 2a1 mRNA, while increasing Runx2 in dead and deformed embryos, without altering Twist. Results suggest that oxidative stress, the UPR and cell death play key roles in SeMet induced deformities and altered skeletal development factors.

273 Identification of biologic binding of uranium in Danio rerio gonads. Impact on their functionality

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Uranium (U) is a radioelement naturally present at trace level ($\mu g.L^{-1}$) in the environment. This level can increase due to geological influences or human activities. Previous waterborne exposures of zebrafish have shown reprotoxic effects after bioaccumulation in the gonads from 20 $\mu gU.L^{-1}$. The objective of this work is to understand this reprotoxicity by studying: i) the U-protein complexes present in gonad (metallomic approach) and ii) the differences in proteome expression between exposed and control groups (proteomic approach). Six groups of adult zebrafishes were studied: waterborne exposed to 0 and 20$\mu g.L^{-1}$ of U for 5 and 20 days, with or without reproduction after 20 days. Bioaccumulation in target organs was performed after exposure. Metallomic approach, i.e. non-denaturing intact uranium-protein complex study, has been developed for our samples. A two-dimensional (pIxMW) fractionation analysis of metals (U, Fe, Cu, Zn and P) was performed using hyphenated separations, i.e. off-gel electrophoresis (OGE) and size exclusion chromatography (SEC) followed by multi-elemental detection (ICP SF MS). Subsequently, identification of potential U protein target was carried out in U-rich OGE fractions by µRPC- ESI FT MS/MS after trypsic digestion. In parallel, phosphorylated protein distribution, as well as vitellogenin amount, were assessed using specific staining or immunodetection after western blot, respectively. Finally, comparison of proteome expression between zebrafish groups was carried out by differential in gel electrophoresis (DiGE). Significant bioaccumulation of U in gonads has been shown with a depuration effect of reproduction event. At the molecular level, whatever the exposure conditions, U mainly coeluted (87±8%) with a 40 kDa-protein of 7-8 pI, also rich in phosphorus. That suggests a strong affinity between U and phosphorylated proteins. In this fraction, potential targets identified included vitellogenin fragments, elongation factors, HSP and SOD. DiGE analysis showed differences between control and U-exposed groups in expression of low molecular weight-proteins that will be further characterized by MS. Cross analysis between both approaches has now to be done to assess if targets are common. Highlighted proteins may be candidates for uranium exposure biomarker and the identification of U ecotoxicity profile. Acknowledgements: Authors acknowledge the NEEDS-Environment program for the funding of TARGETS project supporting this work.

274 Energy cost of metal detoxification strategies in phytoplankton

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Microalgae use various cellular mechanisms to protect themselves against metal toxicity. However, the efficiency of those mechanisms can be limited since metal detoxification often remains incomplete, even at low, non-growth inhibitory metal concentrations. If the energy cost of metal detoxification is high, there could be a trade-off between the cost of detoxifying these metals in the cell and the energy cost of allowing some of these metals to spillover onto metal-sensitive sites (Strategy 1). Alternatively, if the energy cost allocated to metal detoxification is low, phytoplankton could replace or repair proteins that are bound to inappropriate metals with a modest use of intracellular metal detoxification mechanisms (Strategy 2). To gain further insights on the strategy used for metal detoxification in phytoplankton, we performed an extensive bioenergetics analysis of intracellular metal detoxification mechanisms as well as of the energy cost allocated to the detoxification of metal-induced reactive oxygen species. We considered the synthesis of metal-binding peptides, polyphosphate, metal efflux, metal reduction, enzymatic and non-enzymatic antioxidants in wild-type marine and freshwater eukaryotic phytoplankton based on the biochemical mechanisms of each detoxification strategies and on experimental measurements of detoxifying biomolecules in the literature. The results show that at the onset of metal toxicity to growth, all the intracellular detoxification strategies considered required as little as < 2 % of the total cellular energy available for growth. We conclude that phytoplankton generally use Strategy 2 for intracellular metal detoxification.

275 Stream benthic and algal community responses to metals: an evaluation of endpoint sensitivity using stream mesocosms

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Larval benthic macroinvertebrate community responses to metals are often used to assess and predict ecological impairment, however, aquatic insect adults and algal community responses have received less attention. We evaluated both structural and functional endpoints by exposing benthic communities to mixtures of Cu and Zn for 14 d using stream mesocosms. We compared two streams in Colorado with past and present EPA superfund designation, the Arkansas River (AR) and the North Fork Clear Creek (NFCC), respectively. Measured responses included the timing and abundance of emerging adults, algal colonization, community metabolism, and benthic community composition. EC50 values, defined as the metal concentration that reduced the response variable by 50%, were estimated for each endpoint. Results show differences in larval and adult responses within the same taxonomic groups, particularly among midges (Chironomidae) and mayflies (Ephemeroptera); however, algal colonization and community metabolism displayed the greatest overall sensitivity to exposure. The relative sensitivity among the endpoints was also different between the two communities, with the AR exhibiting greater tolerance to metals than the NFCC. This result is likely because of differences in their respective exposure histories and community composition. This research highlights the need to comprehensively assess exposure effects beyond larval macroinvertebrate life stages using structural and functional endpoints that differ in sensitivity, while acknowledging context-dependent responses resulting from different stream assemblages.

Use of Freshwater Mollusk Toxicity Data for Improved Conservation of Water and Sediment Quality

276 The influence of glochidial source; including gravid mussel collection site and year on the sensitivity of glochidia to a reference toxicant (NaCl)

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Unlike, many laboratory-cultured test organisms employed in standardized toxicity tests, the larvae of freshwater mussels (glochidia) are typically obtained from field-collected gravid mussels. While employing naturally-sourced organisms can enhance ecological relevance, wild organisms can potentially introduce additional variability in contaminant response. Because the early life stages of freshwater mussels are among the most contaminant-sensitive aquatic organisms and therefore their responses to waterborne contaminants can influence the derivation of water quality regulations, it is important to understand whether organism source affects toxicity endpoints. This study examined the influence of gravid mussel source, both temporal and spatial differences, on the response of glochidia to a reference toxicant. Acute toxicity tests with sodium chloride and three Lampsilis species were used to determine year-to-year variability in EC50s when mussels were collected from the same sites in Ontario (Canada) for up to eight years. In addition, the influence of gravid mussel collection site on glochidial toxic response was investigating by comparing salt sensitivities of one mussel species (Lampsilis fasciola) collected from different watersheds. Significant differences in a species' EC50 were observed between glochidia obtained from gravid mussels collected from different watersheds. There was less variability in EC50s when gravid mussels were collected from the same location over multiple years. These results illustrate that gravid mussel source can influence the response of glochidia to waterborne contaminants, and that spatial differences (collection site) in organism source had a stronger influence on the toxic response than temporal differences (collection year).

277 Effects of nitrate and an estrogen singly and in combination on freshwater mussel metamorphosis

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Water quality and contaminants have been identified as critical factors for freshwater mussel populations, many of which are highly imperiled throughout North America and the world. Nutrient pollution, specifically nitrate, is one of the most prevalent causes of water quality degradation globally, with increasing anthropogenic input from suburban and agricultural runoff, municipal wastewater, and industrial waste. Additionally there is mounting evidence that nitrate has endocrine-disrupting effects on aquatic organisms. Nitrate often co-occurs with known endocrine disruptors such as hormones, pointing to the need to understand how these compounds may be interacting to affect wildlife. The potential effects of nitrate and hormones to freshwater mussels are largely unknown, particularly during the parasitic stage of the freshwater mussel lifecycle during which metamorphosis from larvae to juvenile occurs. Therefore, we investigated the effects of nitrate singly and in combination with a model estrogen on freshwater mussel larvae (glochidia) viability, attachment success on host fish, metamorphosis success, and timing of metamorphosis. In the first experiment we exposed Lampsilis siliquoidea glochidia for 24 hours to environmentally relevant nitrate concentrations (0, 50, or 250 mg/L NO_3) alone and in mixture with an environmentally relevant concentration of ethynylestradiol (EE2; 5 ng/L) before inoculation on their primary host, largemouth bass (Micropterus salmoides). In a separate experiment, we exposed largemouth bass to the same series of nitrate and EE2 treatments for 14 days prior to inoculating with unexposed L. siliquoidea glochidia to determine if effects of these compounds on metamorphosis are mediated through the host fish. Nitrate and EE2 exposure altered glochidia attachment, metamorphosis success, timing of metamorphosis and number of juveniles produced and the relative influence of glochidia exposure compared to fish exposure will be discussed, along with a comparison to acute toxicity values for these compounds. Results of these studies are important for improving understanding of the effects of these common contaminants on freshwater mussels at the critical stage of metamorphosis and to better understand the role of water quality in assessing habitat suitability for mussel conservation efforts.

278 Does freshwater mussel propagation method influence juvenile chemical sensitivity?

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Freshwater mussels of the family Unionidae are ecologically important and globally imperiled, and research is urgently needed to guide their protection and conservation. Identifying and mitigating chemical stressors is an important part of the process, as is assessing mussel-specific sensitivity to pollutants to establish water quality criteria. The newly transformed juvenile life stage has been shown to be sensitive to certain toxicants and is often used in toxicity testing. Thus, there is a need to transform mussel larvae (glochidia) into juveniles within a laboratory setting. Over the past several decades, conservation aquaculture has significantly advanced propagation techniques and long-term growth and maintenance of propagated mussels. Improving standard host-fish (in vivo) infection techniques has contributed to this success, but recently, in vitro culture methods have made it more efficient and cost-effective to raise juvenile mussels in the laboratory. However, ASTM International cautions against using in vitro propagated juveniles in toxicity tests unless their relative chemical sensitivity to in vivo juveniles is described. The objective of this study was to evaluate the relative sensitivity of juvenile mussels produced from both propagation methods to selected chemical toxicants. We conducted 96-hour acute toxicity tests according to the ASTM International guidelines with three species (Lampsilis cardium, L. abrupta, and Utterbackia imbecillis) and six chemicals: chloride, nickel, ammonia, copper, and aquatic herbicides Clearigate and Nautique. We calculated the median lethal concentration (LC50) for each species-chemical combination and compared the LC50s of the in vitro and in vivo juveniles. Statistically significant differences in LC50 between in vitro and in vivo propagated juveniles were observed in 8 of the 17 trials. Six of the eight statistically different tests for a given chemical were within the intra-laboratory variation that has been demonstrated in a recently published study. Moreover, all of these statistical differences were within the variation for inter-laboratory comparisons for a given chemical in the same study, and therefore, indicate that in vitro propagated juvenile mussels may be appropriate for use in ASTM-based toxicity testing. The differences that we observed were likely related to age, developmental stage, or relative condition of the juveniles, as they appeared to become less divergent over time.

279 Sensitivity of the early-life stages of freshwater mollusks to neonicotinoid and butenolide insecticides

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Neonicotinoid insecticides can be transported from agricultural fields, where they are used as foliar sprays or seed treatments, to surface waters by surface or sub-surface runoff. Few studies have investigated the toxicity of neonicotinoid or the related butenolide insecticides to freshwater mollusk species. The current study examined the effect of neonicotinoid exposure to the early-life stages of the ramshorn snail, Planorbella pilsbryi, and the wavy-rayed lampmussel, Lampsilis fasciola. Juvenile P. pilsbryi were exposed to imidacloprid, clothianidin, or thiamethoxam for 7 or 28 d and mortality, growth, and biomass production were measured. The viability of larval (glochidia) L. fasciola was monitored during a 48 h exposure to six neonicotinoids (imidacloprid, thiamethoxam, clothianidin, acetamiprid, thiacloprid, dinotefuran), or a butenolide (flupyradifurone). The 7-d LC50s of P. pilsbryi for imidacloprid, clothianidin, and thiamethoxam were $\geq$ 4000 µg/L and the 28-d LC50s were $\geq$ 182 µg/L. Growth and biomass production were considerably more sensitive endpoints than mortality with EC50s ranging from 33.2 to 122.0 µg/L. The LC50s for the viability of glochidia were $\geq$ 456 µg/L for all seven insecticides tested. Our data indicate that neonicotinoid and butenolide insecticides pose less of a hazard with respect to mortality of the two species of mollusk compared to the potential hazard to aquatic insects.

280 Toxicity of an Environmentally Relevant Suite of Major Ions and a Trace Element on Juvenile Rainbow (V. iris) and Oyster (E. capsaeformis) Mussels

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The Powell River in Virginia and Tennessee, USA supports diverse freshwater mussel assemblages. Coal mining occurs in the watershed. Throughout the river's extent major ion and trace element concentrations are elevated and mussels have been extirpated or are declining. We conducted a laboratory study to assess the effects of major ions and the trace element nickel (Ni) on growth and survival of juvenile mussels, including one common (Villosa iris) and one endangered (Epioblasma capsaeformis) species. Mussels were exposed to environmentally relevant concentrations of major ions and Ni, to assess the combined toxicity and the potential interaction of Ni with HCO_3(-). Mussels were exposed to diluted pond water alone (control), with Ni only (Ni-control), and with environmentally relevant major ion mixtures with and without Ni for 70 days. The first treatment mimicked low-flow concentrations of Ca(2+), Mg(2+), K(+), HCO(-) and SO_4(2-) in the Powell River at a total ion concentration of 942 mg/L. The second treatment combined the first treatment mixture with an environmentally relevant concentration of Ni (14 µg/L). Mussel survival differed significantly between species, as mean survival was 84.2% for E. capsaeformis and 92.7% for V. iris. There were no significant differences in overall survival between treatments and controls for either species. Total growth showed little variation and was not significantly different between treatments and controls. Results suggest that major ion chronic toxicity alone or in combination with Ni is not the primary source of toxicity for juvenile mussels in the Powell River. The results also suggest that HCO_3(-) does not increase the toxicity of Ni in this system.

281 Chronic sensitivity of freshwater mussels to chemicals with different toxic modes of action

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Native freshwater mussels are among the most imperiled groups of animals, experiencing significant global declines. However, mussels are generally under-represented in toxicity databases. Studies were needed to compile a more comprehensive toxicity database that includes a diversity of mussel species and chemicals to compare the sensitivity of mussels to other freshwater species, and to evaluate the degree to which existing or proposed environmental guidance values and pollutant discharge permit limits are protective of mussels. Over the past 15 years, we have developed, validated, and applied methods to estimate the acute and chronic toxicity of contaminants to freshwater mussels, evaluated the sensitivity of mussels relative to commonly tested fish (e.g., Pimephales promelas and Oncorhynchus mykiss) and invertebrates (Ceriodaphia dubia and Hyalella azteca), and determined the protectiveness of US Environmental Protection Agency (USEPA) 304(a) ambient water quality criteria (WQC) and state water quality standards (WQS) for native mussels. Here we present the results of chronic (28- or 84-day) toxicity tests conducted with juvenile mussels (Lampsilis siliquoidea, L. fasciola, L. abrupta, Villosa iris, and Epioblasma capsaeformis) in water exposures to up to 14 chemicals (chronic nitrate test is ongoing). The chemicals were chosen based on the interest of USEPA or states in developing or updating WQC or WQS and based on different chemical classes and toxic modes of action. Chronic toxicity data from mussels were compared to those from other freshwater species in compiled databases. Mussel species mean chronic values were in the low percentiles (i.e., more sensitive) of species sensitivity distributions (SSDs) for ammonia, chloride, potassium, sulfate, aluminum, chromium (VI), copper, lead, nickel, and zinc, but in the middle and high percentiles of SSDs for cadmium and two organic chemicals (4-nonylphenol and azoxystrobin). Chronic values of some chemicals, including ammonia, chromium (VI), copper, nickel, zinc, chloride, and sulfate, were about equal to or below the chronic WQC or WQS, indicating the WQC or WQS might not protect the mussels tested.

282 Comparing a novel Artificial Mussel and Live freshwater Mussels to Biomonitor Trace Metals

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Mussels are routinely used in biomonitoring trace pollutants in aquatic systems because of their sessile filter feeding lifestyle. In Australian freshwaters only a few species of mussels exist and their population numbers are low. Therefore using live mussels for biomonitoring trace metals in freshwaters is problematic. This study investigated whether a novel artificial mussel (AM) can be used as a suitable replacement for live mussels to monitor trace metals in freshwaters. Both field and laboratory experiments were used to evaluate the efficacy of the AM in comparison with live mussels (LM). Three test sites were chosen in the Goulburn-Murray Water (G-MW) systems Victoria, Australia, comprising river, channel and drain, in conjunction with controlled laboratory experiments at RMIT University. The native freshwater mussel, Velesunio ambiguus was used as the comparative live biomonitor. Both AM and LM were deployed in field trials for three months at the selected locations in the G-MW system. In the laboratory experiments, AM and LM were exposed to varying concentrations (as mixtures) of Cu, Pb and Zn (Cu 20-50 µg/L, Pb 50-200 µg/L, and Zn 100-500 µg/L) over a 28 day period. The live mussels showed preferential uptake of the essential metals Zn and Cu, whereas all trace metals were accumulated without preference by the AM. AM were found to be an effective replacement for native mussels in monitoring the bioavailability of non-essential trace metals in freshwater, however the AM did not reflect the accumulation and hence possibly the bioavailability of essential trace metals by V. ambiguus . Results are reviewed in terms of selective uptake and depuration of essential trace metals by biota when compared with passive samplers including the AM.

283 Imposex of freshwater snail in Eutrophication Lake

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The earlier studies showed that more than 200 marine gastropods were induced to the occurrence of imposex because they were exposed to environmental concentrations of organic toxins such as tributyltin [1], which was primarily through its interaction with RXR pathway [2]. However, rare attention was focused on other agents or freshwater gastropods. In our present study, the imposex of Parafossarulus striatulus, which is a native freshwater snail in China and distribution widely in lakes and ponds, was found after migration into a eutrophication environment for 3 months. In order to find the potential mechanism of the occurrence of imposex, the snails were exposed to TBT, 9cis- retinoic acid (9cis-RA) and M. aeruginosa under control conditions. After 2 months, as expected, the imposex characteristic was found in females, not only in TBT- (100 ng Sn/L) and 9-cis RA (5 mg/L) groups, but also in M. aeruginosa (>10^8 cell/L)exposure group, which was the dominant species in Taihu. Then, the transcriptome and proteome were performed simultaneously to find the potential inner molecular response mechanism although the shortage of bio-information of gastropod. Interestingly, some key molecular signals were found which could be used to speculate the occurrence of imposex.

Birds as Indicators of Environmental Change: Molecular to Population Effects of Contaminant Exposure and Other Stressors

284 Differential Gene Expression Analysis of Endocrine Effects of In Ovo and Dietary 17β-Trenbolone on Japanese Quail Using RNA-Seq

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Endocrine active substances (EASs) such as 17β-trenbolone (17βT – a synthetic anabolic steroid), have been shown to cause reproductive dysfunction in various aquatic and avian species. Previous studies of Japanese quail (JQ) exposed to 17βT, conducted in our lab using qPCR, found significant changes in the expression of a select group of genes along the hypothalamic-pituitary-gonadal-liver (HPGL) axis. To better understand the broader effects of androgens on birds, we used RNA-Seq to investigate changes in hepatic gene expression in JQ embryos and adults exposed to 17βT. Finding genes that are differentially expressed across treatments is an integral part of characterizing the mechanisms of action of EASs. RNA-Seq is a powerful, high-throughput tool that provides a global interrogation of differential expression of transcripts and their isoforms. Hence, the objective of this study was to identify pathways perturbed in embryonic and adult JQ exposed to 17βT. JQ embryos were exposed in ovo and the JQ adults were exposed in ovo and via dietary uptake post hatch to 17βT (1 and 10 ppm). In total, 1194 genes and 2089 genes were found to be down and up regulated, respectively, in male embryos; 304 and 698 genes were found to be down and up regulated, respectively, in female embryos; 20 and 41 genes were found to be down and up regulated, respectively in adult males; 23 and 40 genes were found to be down and up regulated, respectively in adult females. In female embryos, the peroxisome proliferator-activated receptor (PPAR) signaling pathway was significantly affected by exposure to 17βT. This suggests a disruption of metabolic functions, affecting genes related to cholesterol synthesis. The Gene Ontology (GO) enrichment analysis identified genes grouped under cellular components and biological processes as being significantly over expressed in female and male embryos. However, none of the genes identified in this enrichment analyses were directly related to the steroid pathways. Furthermore, in both sexes, the effects did not translate to the adults, indicating that an inherent regulatory mechanism might be compensating for the exogenous stress caused by trenbolone.

285 A slice of knowledge: Toxicological screening of priority compounds using an avian ex vivo slice culture approach

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In vitro screening assays have helped transform toxicity testing of priority environmental chemicals and mixtures. Such assays typically utilize primary cell cultures, immortalized cell lines or cell-free methods. The reliance of these assays upon single cell types, genetically similar strains of cells or a lack of cells altogether introduces challenges in terms of extrapolating results to the whole animal. In this study, liver slices were obtained from late-stage chicken and double-crested cormorant embryos (n=6/species) and exposed to graded concentrations of polychlorinated biphenyl (PCB) 126, the organic flame retardant tris methylphenyl phosphate (TMPP), and a complex environmental extract in 24-well culture plates. The technique combines the throughput of a plate-based toxicity assay with the ability to assess inter-individual variability in an intact liver sample. Species-specific ToxChip polymerase chain reaction (PCR) arrays were used to determine hepatic transcriptomic profiles after 24 hours of exposure. Gene expression responses were compared to those in embryonic hepatocytes of the two species exposed to the same chemicals. Certain pathways (e.g. phase I/II metabolism) responded concordantly between hepatocytes and liver slices. Ultimately, this method improves the biological relevance of our toxicogenomics screening program, thereby permitting the prioritization of resources towards compounds presenting the greatest potential risk to wild avian species. However, it will be necessary to compare these findings to whole animal exposure studies to determine the full utility of this ex vivo screening assay.

286 Novel Brominated Polyphenyl Ether Contaminants Competitively Interact with Thyroid Hormones for Recombinant Avian and Mammalian Transthyretin In Vitro

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Tetradecabromo-1,4-diphenoxybenzene (TeDB-DiPhOBz) is a highly brominated additive flame retardant (FR) and an alternative FR for 2,2',3,3',4,4',5,5',6,6'-decabromodiphenyl ether (BDE-209). There are several known commercial formulations and products containing TeDB-DiPhOBz, however little information is available on the magnitude and global range of its use. With 14 bromine atoms TeDB-DiPhOBz has low bioavailability, however it can undergo rapid photolytic debromination to e.g. tetrabromo- to heptabromo-DiPhOBz. These photodegradation products have increased bioavailability, and toxicological concern is therefore warranted. Interestingly, methoxylated tetra- to hexa-brominated DiPhOBz have recently been identified for the first time in eggs of herring gull (Larus argentatus), a bioindicator species of the Laurentian Great Lakes. These novel contaminants are thus suspected degradation products of TeDB-DiPhOBz, and metabolism studies using gull microsomes confirm that several methoxy- and hydroxy- polybrominated DiPhOBz compounds are formed in vitro. Virtually no toxicological data exist for this BFR or its potential degradation products, however in vitro chicken hepatocyte and avian PCR assays using photodegradation products of TeDB-DiPhOBz report some alterations in mRNA expression (e.g. CYP1A4 involved in the metabolic pathway). The present study investigates the potential thyroidogenicity of tetrabromo-DiPhOBz along with methoxy- and hydroxy- conjugates, using an in vitro competitive protein binding assay with both recombinant avian and mammalian thyroid hormone transport protein transthyretin. Perturbation of thyroid hormone transport is considered to be one mechanism of action that may affect thyroid function, which is a major toxicological concern with PBDEs and especially hydroxy-BDE metabolite compounds.

287 Responses in thyroid parameters of hatchling American kestrels following embryonic exposure to priority flame retardants

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The potential toxicity of priority chemicals is of considerable interest to the Chemicals Management Plan of the Canadian federal government, and the US Environmental Protection Agency. Recent priority chemicals have included 1,2-bis(2,4,6-tribromophenoxy)ethane (BTBPE; CAS 37853-59-1), and two components of the technical mixture, FireMaster 550®, specifically 2-ethylhexyl-2,3,4,5-tetrabromobenzoate (EHTBB also abbreviated ast TBB; CAS 183658-27-7), and bis(2-ethylhexyl)-2,3,4,5-tetrabromophthalate (TBPH; CAS 26040-51-8). While these flame retardants occur in the environment, including in biota such as birds, their potential effects on birds is largely unknown. Following approved procedures, fertile eggs of captive American kestrels (Falco sparverius) were injected with organic safflower oil (controls) or one of three doses of BTBPE, TBB or TBPH in safflower oil into the aircell on Embryonic Day 5 at concentrations within levels reported in wild bird eggs (10, 50 or 100 ng/g ww). The safflower oil solutions of these 3 individual chemicals (10 and 50 ng/g) were prepared by serial dilutions of the stock 100 ng/g dosing solution, and in sufficient volume for all egg injections. Eggs were artificially incubated until hatching. From each hatchling, blood was collected and organ somatic indices (SI) calculated. Total thyroxine (TT_4) and total triiodothyronine (TT_3) concentrations were analyzed in duplicate in plasma and right thyroid glands. The left thyroid glands were assessed for histological changes, and hepatic tissue analyzed for the outer ring deiodination activity transforming T_4 to T_3 (T_4-ORD). Results suggest that histological changes and activation of the thyroid gland occurred in hatchlings of both sexes, but that responses to the different exposure concentrations and chemicals were not consistent between the sexes. Potential changes in these parameters, as well as hatching success and deformities, in relation to the uptake of these chemicals over the course of embryonic development, will be discussed.

288 Relative potency of polycyclic aromatic hydrocarbons (PAHs) in birds; comparisons among congeners and species

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Polycyclic aromatic hydrocarbons (PAHs) are potent contaminants that are broadly distributed in the environment. Birds have the potential to be exposed to high levels of these chemicals, and yet surprisingly few data describing the relative potency of PAHs are available for avian species. Here, we determine LD50 values for 5 PAHs in birds and explore relative potency and sensitivity among congeners and species. Graded concentrations of each congener were injected into the air cell of unincubated chicken (Gallus gallus) eggs. The eggs were artificially incubated at 37.5°C, and the embryos were monitored throughout development and hatching. All of the PAHs that we tested caused dose-dependent increases in embryomortality, but few other abnormalities (e.g. weight changes, deformities, time to hatching) were observed. Critical windows of developmental sensitivity to were identified between embryonic day 3-9 and 16-22. The rank order potency of DahA > BkF > IdP > BaP > BaA corresponded well with previously published data for fish and mammals, and with in vitro measure of the potency of PAHs in birds. For example, ethoxyresorufin-O-deethylase (EROD) EC50 values from cultured chicken embryo hepatocytes were highly predictive of the LD50 values that we generated ($p < 0.001$, $r^2 = 0.99$). In order to assess differences in sensitivity among species, we also injected Japanese quail (Coturnix japonica) eggs with graded concentrations of BkF, and found that sensitivity in chicken and Japanese quail was approximately equivalent. This contrasts with previous studies that suggest that dioxin-like compounds (a related class of chemicals) are many times less potent in Japanese quail than in chicken (e.g. 40-fold difference in sensitivity to 2,3,7,8-tetrachlorodibenzo-p-dioxin). The data presented here contribute to our developing understanding of mechanisms underlying variability in responses to PAHs among congeners and species.

289 Miniaturized passive air sampler: a novel tool to monitor halogenated flame retardants in wild birds

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Atmospheric transport of halogenated flame retardants (HFRs) is now well established. Added in a large range of consumer products to achieve fire safety standards, they ultimately migrate from their commercial substrates to the global atmosphere during their lifespan and beyond. Considering the ubiquitous distribution and potential toxicity of a large array of HFRs, it is crucial to understand their environmental fate and exposure pathways. Previous studies have reported large variations in HFR levels in gulls from the Great Lakes and St. Lawrence River basin. While it is largely accepted that birds are predominantly exposed to HFRs through diet, atmospheric exposure has also been suggested as potential exposure pathway. A recent study from our laboratory suggested that atmospheric exposure might be a determinant exposure pathway for HFRs in ring-billed gulls (Larus delawarensis) from the Montreal area (Canada). However, there is to our knowledge no method allowing the assessment of atmospheric exposure of wild birds to HFRs. The objective of this study was to adapt a passive air sampler (PAS) mounted on free-ranging gulls for monitoring HFR levels in air in both the gas- and particle-phases. Two different adsorbents (polyurethane foam combined to a glass fiber filter versus polydimethylsiloxane) and three exposure periods (one, two, and three weeks) were tested in the field. Results showed that the PAS accumulated all major polybrominated diphenyl ether (PBDE) congeners, hexabromobenzene, Dechlorane 604 Component B, and Dechlorane plus (DP) isomers. Differences in HFR patterns were observed between the two adsorbent types and the three exposure periods. In PAS containing polyurethane foam combined to a glass fiber filter, the contributions of DecaBDE to ΣPBDE were greatest at two weeks of exposure, followed by PentaBDE. DP isomers were the most abundant emerging HFRs determined in these PAS. The larger contributions of DecaBDE and DP were consistent with atmospheric levels reported in the Great Lakes area as well as with tissue levels in ring-billed gulls from Montreal. This novel miniaturized bird-borne PAS thus provided valuable information on the non-dietary exposure of free-ranging birds to ubiquitous HFRs in the environment.

290 Assessing the effects of legacy contaminants on egg and nestling survival of Tree Swallows in Great Lakes Areas of Concern

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Great Lakes Areas of Concern (AOCs) are affected by many stressors, some of which are environmental contaminants including PCBs, PBDEs, persistent organochlorine pesticides, dioxins, benzenes, and other chemicals. These toxicants can accumulate in aquatic biota and ultimately transfer to insectivorous birds that use the aquatic areas within AOCs. We used a relatively new multistate survival modeling approach to examine the relationship between avian egg and nestling survival and 11 contaminant concentrations in representative eggs of Tree Swallows (Tachycineta bicolor) using nest boxes placed near reference (n=10) and contaminated sites (n=59) around the Great Lakes. A total of 1,303 nests and 7,752 eggs were included in the modeling effort. Our analyses controlled for other common sources of variation in egg survival, including female age, date within season, year, and both site and AOC. Site, date within year, year, and female age all proved to be important variables in explaining egg survival. Among environmental contaminants, we found few associations between egg and nestling failure and contaminant concentration in representative eggs. Total dioxin furan toxicity equivalents (TDFTEQ) was significantly positively associated with egg failure, but significantly negatively associated with nestling death. Across the full dose response for this contaminant, empirically observed values of TDFTEQ were concentrated at the low end, with only a few values at the higher end of the dose-response. Site, as an explanatory variable, proved much more valuable, as judged by AIC_c, than AOC, suggesting that sites within AOC can vary considerably in stressors and/or response to stressors. Overall, concentrations of the 11 contaminants examined here, appear to be at concentrations low enough to cause few problems for Tree Swallow reproductive success.

291 Differences in mercury exposure of breeding Leach's storm-petrels related to their foraging patterns in the northwest Atlantic Ocean

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Leach's storm-petrels (Oceanodroma leucorhoa) are small seabirds that breed on coastal islands and make long, multi-day foraging trips during incubation, often beyond the continental shelf. In 2013-15, we attached geolocator tags to adult storm-petrels at seven breeding colonies in Atlantic Canada, to monitor their foraging trips during incubation. When the birds were recaptured 2-4 weeks later, the geolocator data were downloaded and a small blood sample was collected for analysis of total mercury and stable carbon and nitrogen isotope ratios (δ13C and δ15N). Leach's storm-petrels made offshore foraging trips that ranged 260 – 1400 km from their breeding colonies, and foraged over ocean depths ranging 170 – 5700 m. Mean mercury concentrations (± SE) in Leach's storm-petrel blood (n=193) were significantly greater from four colonies around Newfoundland (Baccalieu 1.11 ± 0.05 µg/g, Gull 1.00 ± 0.06 µg/g, Middle Lawn 1.28 ± 0.09 µg/g, and Grand Colombier Islands 1.27 ± 0.05 µg/g) than from three colonies in the Bay of Fundy (Bon Portage 0.65 ± 0.08 µg/g, Kent 0.57 ± 0.04 µg/g, and Machias Seal Islands 0.37 ± 0.06 µg/g). Blood mercury concentrations were positively related to the distance that birds foraged away from their breeding colonies, and to the depth of the ocean over which they foraged. We will discuss the insights provided by data on foraging movements from geolocators and on diet from δ13C and δ15N, for interpreting differences in blood mercury concentrations among and within breeding colonies. We will also assess the toxicological significance of the observed blood mercury concentrations, in light of the declining breeding populations of Leach's storm-petrels at several major colonies in Atlantic Canada. Determining mercury concentrations in the blood of these seabirds, along with their foraging patterns, allows us learn about methylmercury dynamics in offshore ocean foodwebs.

Existing and Emerging Contaminants in Changing Arctic Environments

292 Levels and trends of contaminants in Inuit populations living in northern Canada

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The Canadian Northern Contaminants Program (NCP) is concerned with contaminants that reach the Arctic via long-range transport from source areas around the globe. This presentation provides an overview of human biomonitoring data collected over the past decade from the four Inuit regions of northern Canada (Nunavut, Nunavik, Nunatsiavut, and the Inuvialuit Settlement Region). Contaminants of concern in the North include a large number of persistent organic pollutants (POPs) and metals, including mercury. Levels of contaminants are declining in Canadian Inuit populations that have been monitored over time, although chemical concentrations measured in human tissues will vary depending on a person's age and sex and on chemical depuration rates. Moreover, routes of exposure to contaminants vary across the Canadian Arctic. The observation of higher levels of many POPs and metals in Inuit from northern Canada relative to the Canadian general population is likely related to the amount and type of traditional food consumed by Inuit during their lifetimes, in addition to the region where traditional foods are hunted or harvested. On-going biomonitoring by the NCP permits the assessment of spatial and temporal trends for contaminants in people and their environment, and creates opportunities to identify and measure new chemicals in commerce that might be transported to the Arctic. For example, we predict that a small number of compounds included in the third phase of the Canadian Chemicals Management Plan (CMP) may be reasonably persistent in the environment and undergo only very limited metabolism in higher organisms that may be included in the northern traditional diet. NCP human biomonitoring studies, in conjunction with NCP environmental monitoring results for contaminants in other media, have been used to inform the Stockholm Convention for POPs and the Minamata Convention on Mercury and help Canada to meet its obligations under the CMP. NCP assessments on the state of knowledge for contaminants and human health in northern Canada assist in determining whether risk reduction programs nationally and globally are adequate for the protection of health.

293 Organophosphate flame retardants and plasticizers in the Canadian Arctic

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Organophosphate ester (OPEs) flame retardants and plasticizers have received much attention due to their high levels in indoor air and dust and presence in remote locations including the Arctic. The widespread global distribution of OPEs is believed to arrive through various mechanisms including oceanic transport and air transport in the gas phase and on fine particles. Air, water, sediment and zooplankton samples were collected between 2007-2015 as part of ArcticNet and the Northern Contaminants Program in the Canadian Arctic. Samples were mainly taken from on board the CCGS Amundsen but also at Resolute Bay in the lower arctic and Alert in the high arctic. This coordinated sampling helps us understand how OPEs are introduced into the arctic food web. OPEs most frequently detected in the arctic environment were tri-phenyl phosphate (TPhP), tris(2-chloroethyl) phosphate (TCEP), tris(2-chloroisopropyl) phosphate (TCPP), tris(1,3-dichloro-2-propyl) phosphate (TDCPP) and ethyl-hexyl diphenyl phosphate (EHDPP). Levels of OPEs were very high compared to other flame retardants including PBDEs.

294 Perfluoroalkyl Acids in the High Arctic: A Multi-Decadal Depositional Ice Record

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Perfluoroalkyl acids (PFAAs) are persistent, bioaccumulative compounds found ubiquitously within the environment. PFAAs can be formed from atmospheric oxidation of volatile precursors and undergo long-range transport (LRT) through the atmosphere and/or the ocean to remote locations. PFAA deposition is a result of indirect oxidation and direct transport on atmospheric particles. Ice caps preserve a temporal record of this deposition making them useful in studying the atmospheric trends in long-range transport of PFAAs as well as understanding major pollutant sources and production changes over time. A 15 m ice core representing 38 years of deposition (1977-2015) was collected from the Devon Ice Cap in Nunavut and analyzed for PFAAs. Samples were concentrated using solid phase extraction and analyzed using UPLC-MS/MS. The C4-C13 and C18 perfluorocarboxylic acids (PFCAs), C4, C7 and C8 perfluorosulfonic acids (PFSAs), and PFOSA were all detected in the samples. PFCA and PFSA fluxes ranged from 0.162 to 4.44×10^4 ng m^{-2} yr^{1}. All of the PFCA analytes detected in recent years from 2011-2015 show a decrease in flux. This may be due to melting effects or the phase out by the PFOA Stewardship Program. Even-odd pairs of PFCA homologues were observed for the C6-C11 PFCAs with strong correlations, indicating that these compounds are most likely from indirect formation of precursors. The C2-C4 PFCAs have much higher fluxes than the other PFCAs. A number of CFC replacements (HCFCs and HFCs) are additional precursors to these short-chain acids. Data from NOAA for the global mixing ratios of HCFCs and HFCs shows strong correlations with TFA (C2 PFCA). Devon Ice Cap receives pollutants via atmospheric LRT from both North American and Eurasian sources. Air mass back trajectory analyses allow us to determine the fractions of air masses that originate from source regions to the Devon Ice Cap from across the globe. Ion chromatography analyses of marine aerosol tracers further allow us to determine if Devon Ice Cap has received any PFAA contamination from marine aerosols or solely from atmospheric oxidation. Assessments of deposition, homologue profiles, and air mass back trajectories will improve current understandings of LRT of PFAAs to the Devon Ice Cap. This presentation will examine temporal and homologue trends in atmospheric PFAA deposition and compare to known changes in production as well as previous and current ice cap, lake and ocean water measurements.

295 PCB and organochlorine pesticide accumulation and blubber-depth distribution in an Arctic-invading marine mammal predator

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The spatial and temporal range of killer whales (Orcinus orca) within the Canadian Arctic and Greenland has increased in recent years, coincident with deteriorating Arctic sea-ice conditions. With several Arctic marine mammal species available as potential prey, limited evidence suggests that these invading North Atlantic killer whale populations may be shifting their fish-specialized diet towards one that is richer in marine mammals. Such shifts could lead to higher exposures to biomagnifying contaminants such as polychlorinated biphenyls (PCBs) and organochlorine (OC) pesticides. Killer whales are amongst the most contaminated animals on Earth and face protracted health risks as a consequence of exposure to these contaminants. Virtually all previous killer whale PCB and OC analyses have been performed using the outer blubber layer, obtained through remote biopsy approaches. However, this method may be problematic due to possible heterogeneous distribution of PCBs and OCs within the blubber layers. To address potential health concerns for Arctic-invading killer whales and indigenous Arctic consumers, we measured blubber PCB and OC levels in 10 equal-length blubber sections in 20 individuals harvested in southeast Greenland in 2012-2014. We determined the levels of ∑chlorobenzene, ∑chlordanes, ∑dichlorodiphenyltrichloroethane compounds (∑DDT), ∑endosulfan, ∑mirex, ∑hexachlorocyclohexane and ∑PCB. For individuals analyzed thus far, the highest levels were found for ∑DDT (51.1 ± 3.1 µg g-1 lipid weight) and ∑PCB (44.5 ± 2.7 µg g-1 lipid weight), which far exceeded a 1 part-per-million general threshold level of concern. Levels of ∑PCB and ∑DDT were intermediate between levels reported in fish-feeding killer whales and those that predate marine mammals in earlier studies and other regions. Wet weight contaminant levels varied with blubber depth ($p< 0.05$), but after lipid correction, average contaminant levels were generally similar among layers. Nonetheless, the pattern of PCB and OC levels through the blubber depth appeared to vary among individual killer whales, suggesting that other factors beyond lipophilicity may contribute to PCB and OC concentration variation with blubber depth. On-going analysis of PCB and OC levels in additional individuals will allow for in-depth evaluation of the influence of sex, age class, diet (particularly consumption of Arctic marine mammals relative to fish) on the distribution of these contaminants among blubber layers.

296 Presence and fluxes of legacy POPs and PBDEs in the Western Antarctic Peninsula

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Polybrominated diphenyl ethers (PBDEs) are 'emerged' contaminants that were used as flame-retardants for decades in a wide range of consumer and industrial applications, and are still ubiquitously present in the environment today. Yet little is known about the presence, food web transfer and time trends of PBDEs in the Antarctic. A unique set of >200 biotic samples from the Antarctic was analyzed for PBDEs, including phytoplankton, krill, fish and fur seal milk, spanning several sampling seasons over 14 years. PBDE-47 and -99 were the most dominant congeners determined in all samples, constituting >60% of total PBDEs. In fur seal milk, the higher brominated congeners were becoming more prevalent in later years. A temporal trend was observed for $\sum_{12}$PBDE concentrations in fur seal milk, where concentrations significantly increased ($R^2 = 0.53$, $p < 0.05$) over time (from 2000 to 2014). Trophic magnification factors (TMFs) were calculated for this food web for samples collected in 2010/11; all TMFs were found to be < 1 (range 0.33 – 0.87) indicating biodilution. There is no indication of PBDEs decreasing in Antarctic fur seal milk yet, while numerous studies have reported decreasing trends in the Arctic. This surprising result indicates the need for further research if and when PBDE concentrations in the Antarctic will start declining, too. A comparison between both polar regions suggests a delay of PBDE peak by at least 10 years in the Antarctic. Further insights were gleaned from air and snow samples collected during the austral spring (October – November, 2010) along the western Antarctic Peninsula and analyzed for organochlorine pesticides (OCPs), polychlorinated biphenyls (PCBs) and PBDEs to assess the relative importance of long-range transport versus local primary or secondary emissions. Highest concentrations of PCBs, PBDEs and DDTs were observed in the glacier's snow sample, highlighting the importance of melting glaciers as a possible secondary source of legacy pollutants to the Antarctic. In contrast to other compounds, PBDEs seem to have originated from local sources, possibly the research station itself. Diffusive flux calculations indicated that net deposition is the dominant pathway for PBDEs and chlordanes, whereas re-volatilization from snow (when it melts or metamorphoses) was observed for PCBs and some OCPs.

297 Drivers of Spatial Variability in Fish Mercury Levels in the Dehcho region, NT, Canada

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Leadership and community members in the Dehcho region of the Northwest Territories, Canada, are concerned about levels of mercury ([Hg]) in food fishes. While Lake Whitefish (Coregonus clupeaformis) are generally known to have [Hg] below guidelines, other species, such as Northern Pike (Esox lucius) and Walleye (Sander vitreus), have been the focus of consumption advisories in some lakes. Mercury levels in these species vary widely across lakes within a relatively small geographic area. Previous research has been unable to elucidate the main drivers of among-lake differences in fish [Hg] in this region. From 2013-2015, 8 remote Dehcho lakes were sampled for fish, benthic invertebrates, zooplankton, sediment, and water by a collaborative team that included First Nations and academic researchers. Fish mercury concentrations were related to fish stable isotope ratios, age, size, and growth rates, as well as to a suite of water chemistry and sediment variables. Interim results indicate that size-standardized differences in fish [Hg] among lakes were best explained by concentrations of chlorophyll-a for Walleye (R^2=0.9), and by dissolved organic carbon concentration (R^2=0.6) for Lake Whitefish. For Northern Pike, size-standardized differences in [Hg] were best explained by lake chloride concentrations and age-at-size (R^2=0.93). Intercepts of Hg-del^{15}N relationships for each lake were negatively related to pH, and positively related to concentrations of dissolved methyl mercury in water. These results are discussed in the context of causal mechanisms, consumption advice for northern fishers, and a resulting mercury mitigation strategy initiated by the Dehcho First Nations.

298 Tracing rare earth elements (REEs) in food webs from freshwater, marine and terrestrial ecosystems in the eastern Canadian Arctic

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Rare earth elements (REEs) are a group of 17 elements which include the 15 naturally occurring lanthanide metals, as well as scandium (Sc) and yttrium (Y). REEs have critical applications in many high-tech industries, including electronics, medicine, clean-energy, and agriculture. REEs are now also used in eutrophication management systems for freshwaters. Increasing exploitation of these elements has led to a growing scientific interest in REE ecotoxicology, however the scientific literature remains sparse. Field studies examining the bioaccumulation and food web dynamics of REEs are very rare, and none have focused on northern ecosystems which are potentially vulnerable to REEs enrichment from mining activity. Increasing demand for REEs (and decreasing export from China) has led to the recent development of REE mining projects in Northern Canada. The goal of this field-based study was to evaluate the potential for bioaccumulation and trophic transfer of REEs in freshwater, marine, and terrestrial food webs of the eastern Canadian Arctic. Biological samples were collected in 2012, 2014 and 2015 from marine (mussels, common eiders, seals), terrestrial (lichen, moss, hare, ptarmigan, caribou) and freshwater (invertebrates, zooplankton, fish) ecosystems within a restricted geographic radius around Kuujjuarapik-Whapmagoostui (Nunavik, Quebec). Wildlife harvesting and tissue sampling was conducted by local hunters and organized by the Sakkuq Landholding Corporation through a community-based monitoring project. All samples were analyzed for REEs and stable isotope ratios of nitrogen and carbon (Δ15N, Δ13C). Results show both species- and tissue-specific sensitivity to REE bioaccumulation. Lichens, mosses, snowshoe hares, and freshwater and marine invertebrates (freshwater zooplankton, blue mussels and sea urchins) had the highest levels of REE bioaccumulation, while freshwater fish muscles had low concentrations of REEs. All terrestrial and aquatic vertebrates had very low levels of REEs detected in muscle tissues, whereas concentrations in liver tissues were significantly higher for all species. Variation in REE bioaccumulation will be compared to water, sediment and soil concentrations and the potential for biomagnification in the food chain will also be examined.

299 Increasing mercury, cesium and rubidium in Arctic Char in a lake influenced by permafrost disturbance and climate change

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Many regions of the Arctic are undergoing climate-related changes, such as increased temperatures, altered precipitation patterns, and permafrost degradation, which have altered fluxes of water, sediment, carbon, nutrients, and mercury (Hg) to freshwater environments. West Lake and East Lake, are adjacent, geologically similar watersheds in the Cape Bounty Arctic Watershed Observatory on Melville Island (Nunavut, Canada), which are currently undergoing climate-driven changes at different rates. The West catchment, experienced numerous active layer detachments during the period 2007-2012, while the East catchment has experienced only minor permafrost disturbances. We hypothesize that these alterations will increase the mobilization of Hg and other elements will result in increasing concentrations of Hg in char in West Lake. To investigate this landlocked Arctic char have been collected for analysis of total Hg (THg) as well as 31 elements annually from 2008 to 2015. THg was determined in char muscle by EPA Method 7473 using a Direct Mercury Analyser and 31 elements were determined by ICP-MS analysis. Stable isotope analysis showed that char (N=100) have significantly more depleted $\delta^{13}C$ in East vs West Lake (mean ± SD; -26.86±1.06 ‰ (N=73) vs -24.98±1.44‰ indicative of greater terrestrial carbon inputs to West Lake. Also $\delta^{15}N$ is significantly lower in West Lake char (10.06±0.81 ‰ vs 10.99±0.70‰) suggesting differences in food sources. The combined results from 2008 to 2015 collections show that the West Lake adult char have significantly higher Hg concentrations (0.159 ± 0.08 µg/g) compared to East Lake (0.097 ± 0.04 µg/g). THg has declined significantly in East Lake (7.2%/y) but has increased slightly in West Lake (~0.5%/y). Cesium and rubidium increased significantly in char from West Lake (5.5 % and 4.1% per y, respectively) while declining or showing no trends in East Lake. Arsenic, cadmium, and zinc were present at similar concentrations in both lakes in almost all years and showed no trends. Results for East Lake are consistent with observations of Hg in landlocked char in 4 other lakes of similar size on Cornwallis Island, where declines of -5.5 to -19%/y have been observed since 2005. None of these lakes have significant permafrost disturbance. The higher concentrations and lack of a decline of Hg in West Lake char are consistent with higher inputs of THg to West Lake resulting from extensive permafrost disturbance in the West watershed.

Recent Advances and Trends in Poly- and Perfluoroalkyl Substances Research – Part 2

300 Determination of AFFF Components Using A Multivariate Analysis following an LC-QToF MS Acquisition

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Aqueous film-forming foams (AFFFs) have been implemented in both military and commercial fire-fighting activities to extinguish flammable liquid fuels. However, uses of these formulations have resulted inadvertently in the release of contaminants into the environment due to migration from the site of application. The various formulations of AFFFs consist of numerous fluorocarbon and hydrocarbon compounds1. Characterizing the unique as well as common components of AFFFs that are currently implemented is the starting point to tracking these constituents through various environmental and biological compartments. In this work, seven AFFF mixtures were analyzed using QTof MS in order to obtain full spectral acquisition from which a multivariate analysis approach could be taken to identify unique components within the mixtures. Data was acquired using alternating high and low collision energy states across the full analytical mass range (data independent), such that product ions were also generated (MSE). Samples were diluted in methanol and chromatographic separation performed using an existing liquid chromatography method for the analysis of perfluorinated compounds. Instrumental performance with regards to mass accuracy (Distinctive grouping from PCA plots could be observed for three AFFFs in both modes, with cluster of five of the seven in ESI+ and four of the seven in ESI-. Pool samples, which contain all ions used in the multivariate analysis, were clustered appropriately towards the middle. Investigation of exact mass/retention time pairs strongly associated with all groupings using molecular formulae calculations and ChemSpider database searching resulted in the identification of multiple sulfate, hydrocarbon and fluorinated compounds. Further interrogation of the markers using trend plots to indicate presence and abundance across all the injections yielded additional identifications that were either unique to specific formulations or in some cases common compounds across multiple AFFFs. For those constituents that had a proposed structure, product ion structures were assigned and used as a means to support a potential identification.

301 A New Method for the Analysis of Thirty Poly and Perfluoroalkyl Substances in Maternal Serum

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Poly and perfluoroalkyl substances (PFASs) are a large class of anthropogenic and persistent chemicals, some of which bioaccumulate. The National Health and Nutrition Examination Survey (NHANES) and Biomonitoring California have been monitoring twelve PFASs in human serum for over a decade. We have recently extended our list of chemicals to include additional PFASs, with an emphasis on polyfluorinated compounds and short chain replacements to PFOS and PFOA. Currently, we can measure thirty PFAS compounds in human serum. Our compound list includes eight perfluorinated carboxylic acids (PFCAs), four perfluorosulfonic acids (PFSAs), two perfluorinated phosphonic acids (PFPAs), two perfluorinated phosphinic acids (PFPiAs), as well as ten PFCA precursors/intermediates and three PFSA precursors. Our method consists of a manual methanolic extraction of 250µL of serum, clean-up using ENVI-Carb, and compound analysis by liquid chromatography (Nexera UFLC system, Shimadzu) coupled to a triple-quadrupole tandem mass spectrometer (SCIEX QTRAP 5500 MS/MS system). Measured values for National Institute of Standards and Technology Standard Reference Material No. 1958 (NIST SRM) samples were similar to values for the four certified PFASs (recoveries of 72 – 106%, with standard errors ranging from 9 to 11%). For the other compounds that were not certified in the NIST material, in-house QC serum samples at three different spiked-PFAS concentrations were prepared and tested, with compound recoveries ranging from 48% to 132% and standard errors from 7% to 35%. This method is used to analyze samples for Biomonitoring California studies, to determine levels of these PFASs in Californians, and to help establish temporal and spatial trends. We will present preliminary results of PFAS analysis on archived maternal serum samples, collected as part of the State prenatal screening program. Disclaimer: The views expressed herein are those of the authors and do not necessarily reflect those of the California Department of Toxic Substances Control.

302 Monitoring Human Exposure to Poly- and Perfluorinated Alkyl Substances (PFAS) Using Hand Wipes, Silicone Wristbands and House Dust

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Perfluorinated carboxylates (PFCAs) and sulfonates (PFSAs) are globally detected in human tissues but exposure sources are not well understood. Potential sources include diet, drinking water, dermal absorption, air inhalation and dust ingestion. In particular, dust exposure – via hand-to-mouth contact – has not been well quantified. Of concern is direct exposure to PFCAs and PFCAs, but also indirect exposure to precursors such as the fluorotelomer dialkyl phosphates (diPAPs) and fluorotelomer alcohols (FTOHs). PFCAs & PFSAs are widely detected in house dust, but little is known about FTOHs and diPAPs. The aim of this study was to quantify PFCA, PFSA, FTOH and diPAP human exposure in 40 individuals from Durham, NC, via measurement in house dust, hand wipes and silicon wrist bands. Serum samples were analyzed for PFCAs and PFSAs to investigate correlations with exposure levels. A questionnaire was used to assess relationships between behavior and contaminant exposure. House dust was collected using a vacuum and sieved to < 500 µm. Hand wipes were obtained by soaking gauze wipes in isopropyl alcohol and wiping the hands. Participants wore pre-cleaned silicone wrist bands for 5 days, including during showering and sleeping. The 6:2 FTOH was detected in most house dust samples (91%), whereas the 8:2 FTOH was infrequently detected (11%). This is consistent with the reported industry shift from 8:2 to 6:2 telomer-based products. Similarly, the 6:2/6:2 (100%) and 6:2/8:2 diPAP (94%) were frequently detected in dust, whereas the 8:2/8:2 diPAP was infrequently detected (34%). In contrast, PFCAs and PFSAs were infrequently detected in dust. Similar trends were also observed in the hand wipe and wrist band samples. For example, 6:2 and 8:2 FTOH were detected in 100% of the wrist band samples, but 6:2 FTOH was the dominant congener (geometric mean = 190 ng) followed by 8:2 FTOH (14.6 ng). Also, the 6:2/6:2 and 8:2/8:2 diPAP were detected in 100% and 64% of wrist band samples, with 6:2/6:2 diPAP dominating (geo. mean: 3.8 ng) followed by 8:2/8:2 diPAP (1.1 ng). The 6:2 FTOH (r_s=0.59, $p< 0.01$) and 6:2/6:2 diPAP (r_s=0.80, $p< 0.001$) were highly correlated between wrist bands and hand wipes. These trends indicate that wrist bands and hand wipes are suitable measures of recent exposure for FTOHs and diPAPs. Overall, the study results show that direct exposure via hand-to-mouth contact is low from PFCAs and PFSAs, but indirect exposure (FTOHs, diPAPs) may be important.

303 Temporal trends of perfluoroalkyl substances (PFASs) in pilot whales and children from the Faroe Islands

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Exposure to poly and perfluoroalkyl substances (PFASs) has been associated with adverse impacts on immune health, metabolism and risk of obesity in children. Human exposure to PFASs is thought to be predominantly dietary but the relative contribution from different sources varies substantially across populations. The Faroe Islands are located in the middle of the North Atlantic and the traditional diet of the population includes pilot whale. Here we compare temporal patterns over the last three decades of 17 PFASs in pilot whale muscle to children's sera from the Faroe Islands. We use this analysis to better understand factors affecting the lifetime of these compounds in the marine environment and exposures from marine foods. PFASs in both pilot whale muscle and children's sera from the Faroe Islands have decreased from a maximum ca. 1998-2000 over the past decade but we show that these changes are for markedly different reasons. The drop in pilot whale muscle is driven by a decrease in the precursor, FOSA, whereas declines in children's serum are seen across all compounds. We developed a screening model that shows that the changes of FOSA observed in pilot whales can be explained by dietary ingestion originating from partitioning from the atmosphere. We investigate whether the decline in PFOS observed in children is therefore also driven by a decline in indirect exposure to precursors. To do this we look at trends in PFOS isomer patterns, and the detection frequency of the short-lived precursor FOSA. We found that overtime the percent of branched PFOS has decreased from 49% to 34% and that the frequency of detection of FOSA has declined from 100% in 1993 to 6% in 2012. Taken together this suggests that the observed declines of PFOS in children may be due in large part to decreases in exposure to precursor compounds such as FOSA. To quantify the contribution that pilot whale muscle has to overall PFAS burden in this population we link the pilot whale and human datasets with a PBPK model. We estimate and control for changes in whale consumption with a simple dietary survey and longitudinal MeHg concentrations. The results of this research allow us to make inferences about the shifts in future exposure sources of PFASs for marine food consuming individuals.

304 Toxicity of perfluorinated carboxylic acids to a Zebrafish Liver cell line

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Poly- and per-fluoroalkyl substances (PFAS) are used industrially for their stability and resistance to high temperature and chemical degradation, and many environments across the world exhibit low levels of PFAS contamination. However, the commonly used variant perfluorooctanoic acid (PFOA) drew concern as a toxic and persistent organic pollutant. Therefore, it was replaced by shorter carbon chain homologues such as perfluorohexanoic acid (PFHxA) and perfluorobutanoic acid (PFBA). Because few data exist on the toxicity of these substitutes to aquatic organisms, we examined the toxicity of PFOA, PFHxA, and PFBA on a zebrafish liver cell line (ZFL), a model system useful for both biomedical and aquatic environmental studies. Cell viability after 96 h of exposure was lowest for PFOA and PFO-, while PFHxA and PFBA were less toxic. Based on the structural similarity of PFASs to fatty acids, we suspected that these compounds interfere with lipid transport and metabolism. However, we did not detect changes to gene expression of ppar-α,β,γ, fabp3 and crot after 96 h exposure to up to 10 ppm of PFOA, PFHxA, or PFBA. Conversely, we found obvious intracellular lipid droplet accumulation only in PFBA-exposed cells. To examine additional effects on metabolism, we conducted Fluorescence Lifetime Imaging Microscopy using the naturally fluorescent coenzymes NADH and FAD. The fluorescence lifetimes of NADH and FAD and the bound:free ratio of each of these coenzymes decreased in a dose- and carbon length-dependent manner, suggesting disruption of cell metabolism; these data are consistent with an overall decrease in metabolic activity and a partial shift in cellular ATP production from oxidative phosphorylation to anaerobic glycolysis. In conclusion, this in vitro study using ZFL cells revealed that PFASs with shorter carbon chains are less toxic than PFOA, but that exposure to PFHxA or PFBA still affects cell metabolism. Therefore, the application of shorter-chain alternatives is not without risk.

305 Evaluation of PFHx- Pharmacokinetics in Mouse, Rat, Microminipig, Pig, Monkey and Human

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Perfluorohexanoate ($PFHx^-$) elimination kinetics have been determined from published data for multiple mammalian species: mouse, rat, microminipig, pig, monkey and human. For each species, the data was compiled and modeled to identify the kinetic profile of elimination from blood including calculation of elimination rate (hr^{-1}) and elimination half-life (hr). The study shows that $PFHx^-$ elimination kinetics in mammals is consistent with a rapid initial (alpha) phase followed by a slower terminal (beta) phase. Further, the $PFHx^-$ alpha phase elimination has a first-order half-life range of 1-2 hours in mouse, rat, microminipig, pig, monkey and human. The alpha phase elimination in mammals accounts for over 99.7% of the total $PFHx^-$ elimination in mammals. $PFHx^-$ elimination is extensive in the rapid alpha phase and there are no significant pharmacokinetic differences across mammals: mice, rats, monkeys, pigs and humans. The overall conclusion of this kinetic assessment is that $PFHx^-$ does not appear to exhibit significantly different elimination kinetics across a wide range of mammalian species. Details of the assessment for each species will be presented and summarized.

306 Perfluoralkyl Substances at Superfund Sites

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Perfluoroalkyl substances (PFAS) have been recognized as emerging contaminants of concern by State and Federal entities for a number of years. Because of the valuable properties of PFAS, they were used in a wide variety of industrial and commercial products. Some of the known uses are coating on surfaces to impart non-stick properties or stain resistance, dust suppression for metal plating, and aqueous firefighting foam (AFFF). As a result, PFAS may have been released into the environment during manufacturing, application, or disposal of wastes and end products. PFAS have been detected in various environmental media including groundwater, soil, and biota at areas associated with their manufacture, use, and disposal. Sites contaminated with PFAS are emerging with high visibility to the public. However, the environmental fate and transport as well as the toxicology of the various PFAS compounds is not completely understood, and analytical challenges still exist. This talk will focus on specific PFAS, media, and site types where PFAS, the challenges site managers have encountered in the field; and additional research that is needed to improve our understanding of PFAS toxicology and analytical methods.

Systems Modeling Approaches for Ecotoxicology to Link Molecular Responses to Ecosystem Effects

308 Linking the Adverse Outcome Pathway to Dynamic Energy Budgets: A conceptual model

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Ecological risk assessment quantifies the likelihood of undesirable impacts of chemicals and other stressors at higher levels of biological organization. In practice, the need for methodology that is cost-effective and minimizes the use of vertebrate animals has and will increasingly limit available data to individual organisms and suborganismal processes. Two ecological modeling approaches are being used to connect information at different levels of organization. The first uses quantitative adverse outcome pathway (qAOP) models that link molecular initiating events to key events that are relevant to the physiology and/or behavior of the individual organism. The second uses dynamic energy budget (DEB) models that describe the assimilation of energy by an organism and its allocation to growth, development, maintenance and reproduction at all life stages. qAOPs have clear connections to molecular biology and biochemistry, but typically simulate complicated physiological mechanisms with many parameters and are concerned with toxicological endpoints related to a single process (i.e., growth or reproduction); DEB models have weaker connections with specific sub-organismal processes but simulated mechanisms are simpler and can connect endpoints from multiple physiological processes. As members of a working group at the National Institute for Mathematical and Biological Synthesis (NIMBioS), we are exploring approaches to link the two modeling approaches. First, consistent with a DEBtox approach, we connect internal toxicant concentrations to abstract quantities such as “damage inducing events” that impact measureable physiological responses of organisms. Then, we use qAOP key events to either represent or provide direct measures of damage inducing events that manifest at the level of organs or the entire organism. Finally, by linking the qAOP and DEB approaches and then embedding DEB models into individual-based population and other ecosystem models, we narrow the mismatch between available data and ecological outcomes of concern. Our working group is using two case studies (invertebrate and fish) to demonstrate proof of concept and develop a general framework for future model development, evaluation, and communication that can be applied across different levels of biological organization and ecotoxicological endpoints. We are also partnering with another NIMBioS group that is using ecological models (with DEB modeling) to link individuals to ecosystems services.

309 A systems modeling framework to link organism-level effects of chemical stressors to effects on ecosystem services

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Although protection goals for the ecological risk assessment of chemicals are increasingly being framed in terms of ecosystem service delivery, the type of data collected to assess risk is generally at the level of individual organisms. Currently, extrapolation from what is measured to what we want to protect uses overly simplistic approaches (e.g., hazard or risk quotients) that are not solidly grounded in biology or mathematics. This presentation describes progress and ongoing efforts from a working group funded by the National Institute of Mathematical and Biological Synthesis (NIMBioS) to develop a systems modeling framework to link organismal effects to changes in ecosystem services. The proposed framework quantitatively links physiological energetics, to organism performance, population dynamics, and ecosystem service delivery. The working group is applying the framework to case studies involving trout and daphnids. These species were chosen because they are commonly used in EPA and OECD-approved toxicity tests, are ecologically relevant, and yield a range of ecosystem services. We provide an overview of the specific ecosystem services provided by each species and the modeling tools currently being developed and refined. For trout we are focusing on the services delivered primarily through angling and are using individual-based, bioenergetic models to explore the impacts of 17-alpha-ethinylestradiol on a range of organismal endpoints linked to ecosystem services. Daphnids, alternatively, yield indirect ecosystem services such as water clarity that enhances recreation in lentic systems. Currently, the working group is exploring the use of AQUATOX to link data on effects of chlorpyrifos on daphnids to system-level endpoints including phytoplankton abundance which relate directly to water clarity. Importantly, the efforts reported here are designed to dovetail with an additional, coordinated NIMBioS working group focused on a modeling framework that links suborganismal (metabolic, cellular, and molecular) responses to chemicals to effects that manifest at the organism level. The overarching goal of these collaborative, multidisciplinary groups is a generalizable modeling framework and proof of concept that can be used to relate toxicant responses at levels of organization that are more easily studied (sub-organismal, organismal) to outcomes relevant to society and effective environmental management (ecosystem services).

310 : Role of adverse outcome pathways in developing computational models for regulatory toxicology

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Regulatory toxicology for both human health and the environment increasingly is moving from a sole reliance on direct observation of apical toxicity outcomes in whole organism toxicity tests, to predictive approaches in which unacceptable outcomes and risk are inferred from mechanistic data. The adverse outcome pathway (AOP) framework has emerged as a key tool for organizing and communicating knowledge that supports such inference. In this context, AOPs also could support the systematic organization of knowledge to inform and help direct the design and development of predictive computational models to further enhance the utility of mechanistic data for chemical assessment. An international expert workshop exploring this premise was held in September, 2015 in Ispra, Italy. Outcomes from this workshop featured illustrative case studies of AOP-informed model development and application to the assessment of chemicals for skin sensitization in humans and endocrine disruption in fish. The role of problem formulation, not only as a critical phase of risk assessment, but also as guide for both AOP and complementary model development was elaborated. Finally, a proposal for actively engaging the modeling community in AOP-informed computational model development through crowd-sourced competitions was proposed. This presentation will provide an overview of the workshop outcomes relative to a vision for how AOPs can be leveraged to facilitate development of predictive computational models to help support the next generation of chemical safety assessment. . The contents of this abstract neither constitute nor necessarily reflect USEPA, US ACE or European Commission policy.

311 Scaling the effects of multiple stressors from molecular to population levels using Daphnia magna as a model organism

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Understanding the effects of multiple stressors is one of the biggest challenges facing freshwater systems.Although toxicity standards and bioaccumulation models most often consider individual contaminants, most environmental disasters involve complex mixtures of contaminants and other stressors. Because it is impossible to screen for the effects of every possible stressor combination using standard methodology, we are developing a new framework which is not stressor-specific and which will allow for the translation of effects of stressors seen at the subcellular level to larger scale, longer-term impacts at the population and ecosystem level. We employ two quantitative ecological modeling approaches to connect information at different levels of organization. The first uses adverse outcome pathway (AOP) models that link molecular initiating events to key events that are relevant to the physiology and/or behavior of the individual organism. The second uses dynamic energy budget (DEB) models that describe the assimilation of energy by an organism and its allocation to growth, development, maintenance and reproduction at all life stages. Linking the approaches will allow for the screening of thousands of potential stressors on thousands of species, but will require empirical data on a few species to validate models and identify appropriate assays to use for risk assessment methods. In the present study, we used Daphnia magna as a model organism to identify critical data gaps that are needed to link molecular responses to dynamic energy budget processes related to growth, reproduction and reserve dynamics. We exposed individual daphnids to a complex mixture of contaminants found in coal ash, which has recently been the focus of regulatory actions and ecological risk assessments due to environmental spills. Daphnia were were exposed to a gradient of food rations and contaminant concentrations over time. We evaluated gene expression and contaminant body burdens at key life history and molt cycle stages as well as parameters relevant for DEB modeling (weight, length, reproduction, etc) in daphnids exposed to different food and contaminant treatments. These experiments represent a critical first step to bridging the gap between molecular-level responses and larger scale, longer-term impacts at the population and ecosystem level.

312 Linking Adverse Outcome Pathway Analyses to Dynamic Energy Budgets: A Case Study with Endocrine Disruptors and Fish

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Quantitative Adverse Outcome Pathway (qAOP) analyses and Dynamic Energy Budget (DEB) models have complementary strengths. Whereas qAOPs include detail about molecular toxicity mechanisms, DEB models provide quantitative means to project toxic effects observed at the organismal level to impacts expected at the level of populations and beyond. Our overarching goal is to integrate both approaches to improve the predictive power of ecological risk assessment. In this presentation, we show how qAOPs can be embedded within the standard DEB modeling framework. Our examples focus on endocrine disruptors of the reproductive cycle in fishes, notably fathead minnow and various species of salmonids. In a DEB framework, toxicant exposure leads to changes in one or more of the rates at which organisms acquire resources and expend the energy and nutrients therein for maintenance, growth, development and reproduction. Standard DEB considers the workings of hormonal and other forms of regulation in a healthy animal to yield homeostasis, during which the allocation of energy and nutrients to the various budgets are simple functions of animal size and reserve content. In particular, barring severe stress conditions, the partitioning of resources to somatic functions on the one hand and to those supporting reproduction and development on the other is quite simple: the ratio of the two remains constant throughout ontogeny. However, this rule of resource partitioning is too crude for our purposes, as it cannot account for temporal patterns in gonad development during the reproductive cycle of fishes. Moreover, by having control mechanisms implied, standard DEB cannot directly deal with process disturbances due to the toxicant action of endocrine disruptors. Therefore, we have refined DEB theory to include feedback of developmental and reproductive parameters, such as egg mass and liver vitellogenin content, to the allocation of resources to somatic, developmental and/or reproductive processes. This feedback is mediated through hormonal control mechanisms and, thereby, serves as a gateway for linking qAOPs of the impact of endocrine disruptors to DEB.

313 New and emerging statistical machine learning methods for multi-omics data: toward quantitative, molecular ecotoxicology

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Epigenetic and metabolomic information in multiple species responding to matched chemical stimulus has the potential to reveal the evolutionary trajectories of adaptive and eco-responsive regulatory networks in metazoans. To date, in both toxicology and pharmacology, and indeed all areas of exposure biology, model organisms have been used as “surrogates” for target species -- if they respond badly we assume the human or ecological response will be qualitatively similar. This does not always appear to be the case – around 20% of presumed carcinogens show no epidemiological evidence of carcinogenicity. We need quantitative models for ecological and human fitness. To bring about the era of quantitative model systems, we require massive collections of well-designed multi-modal omics studies, and computational methods that are able to seamlessly handle heterogeneity in enormously high-dimensional data, even when we have no hint as to the presence or structure of homogeneous processes a priori. The Consortium for Environmental Omics and Toxicology (CEOT) is in the process of taking the first steps toward a phylogenomically informed, quantitative exposure biology, and this progress is rapidly driving new advances in biostatistical machine learning. In this talk, I will discuss new procedures for feature selection and discovery that we are constructing for use in CEOT, the biological insights they have yielded, and their implications for the future of ecotoxicology. I will discuss the

evolutionary trajectories of environmentally responsive genes, the role of the microbiome in mediating the effects of chemical exposures on host fitness, and outline a conceptual framework by which to design and learn from ecotoxicological exposures.

314 Can standard toxicity tests be used to support population level risk assessments for fish

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Under the Federal Insecticide Fungicide Rodenticide Act (FIFRA), standard toxicity tests are required for fish. Although these studies are focused on individual level effects, they may be used as input parameters to population models in assessing impacts to survival and fecundity. Population modeling in fish is complicated because they exhibit indeterminate growth and other vital rates such as fecundity and survival depend upon size. Because of this, growth estimates are critical for accurate modeling. For assessing mortality from acute exposures, toxicity studies are conducted with standard species (rainbow trout, bluegill) to estimate 96-hour median lethal concentrations (LC50s). For chronic effects, a 30d Early Life Stage (ELS) test is conducted, typically with fathead minnow, in order to identify growth-based no effect levels. In limited cases, full lifecycle test data may be available, to provide information on reproductive effects; however, variability in the fecundity endpoint in some lifecycle tests may be quite large, making interpretation of test results difficult. As an alternative, the Medaka Extended One Generation Reproduction Test (MEOGRT), may offer more power and more consistent estimates of pesticide effects on fecundity. We describe a series of experiments designed to evaluate the ability of standard toxicity tests to adequately provide information on growth, survival and fecundity of medaka (Oryzias latipes) and fathead minnow (Pimephales promelas). We found a strong dependence of fecundity on size, where reductions in fecundity could be induced either through limited ration or chemical stress. Further, medaka exposed to limited ration and chemical stress appeared to allocate differentially to reproduction depending on the amount of energy the animals had available for reproduction. Our results strongly suggest that the recent move towards dynamic energy budget models could provide a unifying underpinning for modeling population-level outcomes from standard FIFRA toxicity tests. However, successful implementation of the approach may require modification of test designs.

315 Quantitative Methods for Linking Adverse Outcome Pathways and Population Models

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In a complex and changing environment (e.g., in the face of climate change) and with an increasing emphasis on sustainability of coupled human-natural systems, reductionist approaches to environmental management that fail to consider interactions, multiple stressors, and spatial and temporal characteristics of exposures and populations no longer suffice. Chemical risks and effects research has traditionally focused on adverse biological effects of chemical exposure to individuals. A more comprehensive assessment of ecological risk is needed to link chemical effects on individuals to those at increasing levels of biological complexity and to evaluate the spatial and temporal context in which chemical exposures occur. An integrated understanding of species activities (e.g., migration), physical stressors (e.g., habitat, climate, etc.) and biological factors (e.g., trophic interactions) is required to link individual-level exposures to population-, community- and ecosystem-level consequences. Most adverse outcome pathways (AOPs) in the literature are qualitative rather than quantitative – here we demonstrate how existing data and models can be integrated through a Bayesian Relative Risk (BN-RRM) framework that explicitly links molecular initiating events to regulatory outcomes of interest and incorporates the influences of multiple stressors. The flexible approach allows multiple stressors linked to multiple outcomes based on integrating existing data and underlying process models. We provide several examples of ongoing case studies – one for a legacy contaminant with a rich database and existing AOP based on acetylcholinesterase inhibition in fish, and another with a less well understood AOP based on immunotoxic effects of pefluorinated compounds.

Alternative Approaches to Animal Testing for Ecotoxicity Assessments – Part 2

316 Assessment of genotoxicity and carcinogenic cell transformation using a tissue regenerating self-signaling cell line from fish

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A pluripotent cell line with tissue regenerating capacities has been isolated from the carp brain. The gene expression pattern of this permanent cell culture has been shown to be very similar to embryonic stem cells of vertebrates. A variant derived from this cell line transgenic for H2B-GFP was established (KCB_{GFP}, DSM ACC3285). The stable fluorescence signal related to the nuclear structures of these cells reflects impacts on the integrity of the genome in a self-signaling manner. KCB-GFP cells are perfectly suited for detecting cytogenetic abnormalities, particularly those caused by genotoxic noxa. Especially micronuclei (MNi), the major endpoint in genotoxicity testing, can be visualized and quantified without fixation and nuclear staining. A dose-dependent induction of MNi could be shown for clastogens as well as for aneugens. Cytotoxic exposure concentrations can be identified by apoptotic / necrotic nuclear patterns such as pyknotic or fragmented nuclei. In contrast to mammalian cell lines used in in vitro toxicology, KCB cells are still susceptible to carcinogenic transformation, since they are not immortalized or tumor derived. Changes in the nuclear fluorescence pattern can be monitored in post-exposure follow up-, or continuous exposure cultures. Preliminary results indicate that transformation to cancer cells – in terms of uncontrolled proliferation and/or alterations of nuclear patterns – may be investigated in the same experiment as MNi evaluation. Preliminary results of implantation experiments have shown that KCB-GFP cells integrate into various tissues in most cyprinid species without teratome formation. The application of molecular markers from cancer research, tumor diagnostic and stem cell biology would aid in the recognition of adverse outcome pathways. Transplantation of cells potentially transformed due to exposure to carcinogens would enable evaluation of teratogenicity in vivo, avoiding systemic exposure of animals. Thus, decisions regarding higher tier testing could be supported by a broader spectrum of in vitro endpoints, and animal testing could be limited to the essential minimum according to the 3R principles.

317 The Chemical Exposure Toxicity Space graphical display of exposure times, aqueous concentrations, and chemical activities for aquatic toxicity tests

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We introduce a novel graphical Chemical Exposure Toxicity Space (CETS) diagram designed to illustrate plausible test conditions for aquatic toxicity tests for fish. The CETS plot is designed to aid selection of chemical concentrations in water, test durations, and for interpreting test results. The CETS plot depicts the solution to the differential equation describing uptake kinetics of a chemical by a one-compartment fish under dynamic conditions, relating aqueous chemical concentration to time and ultimately to the onset of toxicity. The onset of toxicity is determined either by an assumed lethal concentration (LC50) or by the incipient lethal aqueous concentration deduced from a bioconcentration factor and an estimated critical body residue. The plot includes the maximum test duration which is dependent on the test organism and conditions selected. The exposure time is converted to a dimensionless time quantity, Φ calculated as (1-exp(-(k_2)t). LC50 is plotted against Φ and the resulting curve shows the toxic endpoint at a given water concentration or activity for a given Φ. The solubility and maximum test duration parameters become boundaries of the feasible toxicity test conditions. The CETS plots demonstrate differences in toxicity between chemicals as well as how various factors including hydrophobicity and aqueous solubility of the chemical affect the plausible test duration. Hydrophobic chemicals are shown to have a small range of plausible test conditions. For some chemicals such as super-hydrophobic siloxanes no feasible test zone exists. The plots also suggest that a valuable bioassay approach is to establish a constant exposure concentration in advance using passive dosing techniques and measure time-to-death as was done by van der Heijden et al. (2015).

318 Morphological and metabolic characterization of the rainbow trout intestine grown in vitro with a specific focus on the pyloric

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The present study focuses on the isolation of cells from the pyloric region of the rainbow trout Oncorhynchus mykiss and their successful culture and growth using a modified enzymatic dissociation protocol and a larger cohort of fish (>25cm) than conventionally used in research. While not presented herein, this protocol with small modifications can also be successfully applied to other regions of the intestine in addition to smaller size categories. Epithelial cells extracted using this gentle method do not require external substratum to attach to tissue culture treated plates/flasks, although minimal improvement is observed with coatings of fibronectin or collagen. Cell viability was consistently in the range of 90-97% using the outlined procedure with cells visibly attaching within 3-4 hours of extraction. Epithelial colonies were visible after 48 H post first half media exchange and retain their island like morphology for approximately 5 days before gradually spreading into a flattened formation. Mucolytic cells indicative of active transport pathways stained positively on the extracted and attached cells for neutral (strongly) as would be expected in this size category. Immunohistochemical staining for tight junction proteins such as ZO-1 and E-cadherin revealed extremely similar staining profiles between the native tissue and the in vitro grown cells. Attachment success in transwell insert cups is variable between manufacturers with the conventional cup providing the most consistent adherence and growth. Transepithelial resistance measurements (TEER) indicated a comparable resistance in transwell inserts cups to those reported in the literature for fish intestine, however does not come close to what is reported in the mammalian TEER literature. Interestingly, TEM analysis of cells cultured on inserts show comparable profiles to mammalian models and modifications to this system may be required to improve comparability to native tissue. Finally, metabolic activity was assessed through the EROD assay for CYP1A activity and ECOD assay for CYP3A using appropriate relevant control inducers and inhibitors such as BNF, αNF and Rifamapicin. The maintenance and the propagation of the gut model over an 8 week period provide for in vitro fish studies a preliminary step in the minimization of animals necessary for short term dietary exposure studies in addition to a more comprehensive system to investigate co-culture response with different organs.

319 High Throughput Screening Techniques Once Put on the Shelf, Now Ready for Practicality

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One of the biggest challenges when cleaning up sites has always been the question: “will this actually hurt the animals living here?” Testing is performed according to regulatory criteria, and sometimes at great cost to the end user in instances where the source material is unknown. Performing traditional testing can sometimes be a guessing game and a gamble, if the offending analytes are not readily identifiable. In vitro testing has been employed for decades but has not often been utilized in the past during reclamation and remediation activities. This study’s purpose was to bring some of these in vitro tests out of obscurity and evaluate the potential for synergistic and additive effects to organisms (in a soil matrix) using the same methodologies as the USEPA’s ToxCast program, employing high throughput screening in vitro assays. The main criticism in the past of this type of toxicological testing has traditionally been that it is being performed on discrete analytes, not complex mixtures as they are found in the natural environment, and that it has involved live organism studies that are expensive, take a lot of time, and can only focus on acute toxicity. Now, a new study has been performed and validated to employ a suite of screening protocols employing four essential potential pathways that includes (a) the Tui protein neurotoxicity assay, (b) the Muta-Chromoplate for mutagenicity, (c) OxiSelect™ Oxidative DNA Damage kit and (d) MC7F test performed on human mammary gland tissue from a metastatic cancer site, for endocrine disruptors. This is a peer-reviewed, legally defensible tool for risk assessments and are particularly useful in situations such as oil sands reclamation sites, spill events, and forensic investigations and sites involved with unknown complex mixtures. This method is a cost effective tool that can add a lot more value than simple discrete testing of analytical parameters or traditional toxicology techniques.

320 Behavioural toxicity assessment of silver ions and nanoparticles on zebrafish using a locomotion profiling approach

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Zebrafish (Danio rerio) is not only a widely used species in the Fish Embryo Toxicity (FET) test but also an emerging model in behavioural ecotoxicology. By using automatic behaviour tracking technology,locomotion of developing zebrafish (ZF) larvae can be accurately recorded and potentially used in anecotoxicological context to detect toxicant-induced behavioural alterations. In this study, we explored if and how quantitative locomotion data can be used for sub-lethal toxicity testing within the FET frame-work. We exposed ZF embryos to silver ions and nanoparticles, which previously have been reported to cause neurodevelopmental toxicity and behavioural retardation in early-life stages of ZF. Exposure to a broad range of silver (Ag+ and AgNPs) concentrations was conducted, and developmental toxicity was assessed using FET criteria. For behavioural toxicity assessment, locomotion of exposed ZF eleutheroembryos (120 hpf) was quantified according to a customised behavioural assay in an automatic video tracking system. A set of repeated episodes of dark/light stimulation were used to artificially stress ZF and evoke photo-motor responses, which were consequently utilized for locomotion profiling. Our locomotion-based behaviour profiling approach consisted of (1) dose-response ranking for multiple and single locomotion variables; (2) quantitative assessment of locomotion structure; and (3) analysis of ZF responsiveness to darkness stimulation. We documented that both silver forms caused adverse effects on development and inhibited hatchability and, most importantly, altered locomotion. High Ag+and AgNPs exposures significantly suppressed locomotion and a clear shift in locomotion towards inactivity was reported. Additionally, we noted that low, environmentally relevant Ag+concentrations may cause subordinate locomotive changes (hyperactivity) in developing fish. Overall, it was concluded that our locomotion-based behaviour-testing scheme can be used jointly with FET and can provide endpoints for sub-lethal toxicity. When combined with multivariate data analysis, this approach facilitated new insights for handling and analysis of data generated by automatized behavioural tracking systems.

321 Capability and capacity of zebrafish embryos (Danio rerio) to biotransform polar chemicals such as clofibric acid, metoprolole and carbamazepine

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The zebrafish embryo is a popular test organism in ecotoxicological studies increasingly used in toxicological and pharmacological research. Beside the known sensitivity of the zebrafish embryos to xenobiotics such as pharmaceuticals not much is known about the internal concentration of parent substances and respective transformation products (TPs). Biotransformation within the embryo would affect the toxicological or pharmacological dose via the change of concentration- time and effect profiles of the compounds (toxicokinetics & toxicodynamics). This study focuses on the biotransformation of the pharmaceutical compounds clofibric acid (lipid lowering clofibrate metabolite), metoprolole (ß-blocker) and carbamazepine (antiepileptic drug) by zebrafish embryos at different life-stages up to 96 hours post fertilization. We established a screening method based on LC-high-resolution mass spectrometry using state-of-the-art data evaluation to support ecotoxicological and pharmacological studies. This approach is capable of analysing a multitude of polar compounds (Brox et al. 2014). In the presented work this approach is used for the analysis of the 3 test compounds and their TPs. Identification of the compounds was based on the exact masses, isotopic pattern and the specific fragmentation. Known and previously unknown phase I, phase II TPs and even a possible transport (phase III reaction) could be observed. One of the detected and identified 18 TPs of clofibric acid is especially interesting as this phase I product to our knowledge has never been reported before in other biological or technical systems (Brox et al. 2016). For metoprolole all TPs reported from human studies were also detected in the zebrafish embryos. In addition, quantitative data of the metoprolole tests suggest that the transformation capacity of the zebrafish embryos is limited as the exposure to a high dose of metoprolole did not increase biotransformation product quantity compared to a parallel low dose of the same substance. The carbamazepine analysis did show a very high transformation of carbamazepine into acridine. This result would point to a possible underestimation of carbamazepine uptake by zebrafish embryos if it would be the only substance analysed. The results of this study outline the high potential and capability of zebrafish embryos to biotransform xenobiotica. The similarities and differences to other toxicological test systems will be discussed.

322 Cardiovascular and neurodevelopmental metrics as sublethal endpoints for the fish embryo toxicity test

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The United States requires that whole effluent and chemicals be tested for aquatic toxicity using the fathead minnow larval growth and survival (LGS) test. While the LGS test has been effective for determining acute and chronic aquatic toxicity, a fathead minnow fish embryo toxicity (FET) test has been proposed as a more humane alternative because younger organisms are thought to experience less stress during toxicant exposure. Presently, the FET test protocol does not include endpoints that allow for the prediction of non-lethal adverse outcomes or chronic toxicity, which limits its utility relative to other test types. This study investigated the usefulness of sublethal endpoints related to cardiovascular function and development (e.g., heart rate and pericardial area) and neurodevelopment (e.g., eye development and activity levels) and relative expression levels of genes related to growth and development, as additional FET test metrics. FET tests were run with four model toxicants: 3,4 –dichloroaniline, sodium chloride, cadmium chloride, and a water-accommodated fraction of engine oil. Activity levels were evaluated at 24 hours post fertilization (hpf), while eye size, pericardial area, and heart rate were evaluated at 96hpf. Hatched larvae were sampled at the conclusion of the tests (120hpf) for gene expression analysis. Toxicant-induced alterations in these endpoints suggests that sublethal endpoints related to cardiovascular and neurological function and morphology may be useful for estimating predicting non-lethal adverse effects and chronic toxicity. Future studies aimed at linking alterations in these endpoints to longer term adverse impacts are need to fully describe the predictive power of these metrics in whole effluent and chemical toxicity testing.

323 Zebrafish Metabolomics: a Novel Approach for Characterizing Sub-lethal Responses to Xenobiotics

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The introduction of the European Union's Regulation on Registration, Evaluation, Authorization and Restriction of Chemicals (REACH) has placed an increased demand on the use of animals for toxicity testing. At the same time, sensitive and quantitative protocols for characterizing low-dose effects are needed to meet the requirements of 21st century chemical hazard assessment. The high level of conservation of genomic and functional pathways between fish and mammals makes teleost an effective vertebrate model in toxicological testing. Recently the Organization of Economic Cooperation and Development (OECD) recognized the Zebrafish Embryo Toxicity assay (TG236) as an alternative to adult fish toxicity testing. In the present work, we hypothesized that xenobiotics can produce highly specific biochemical fingerprints in organisms exposed at environmentally relevant concentrations. To test this, we measured the metabolomic perturbations in zebrafish (Danio rerio) embryo/larvae following 24-hour exposure to 13 individual chemicals covering a wide range of contaminant classes. Measured metabolites (208 in total) included amino acids, biogenic amines, fatty acids, bile acids, sugars, and lipids. 96 to 120 hours post-fertilization stage, which falls outside of regulatory frameworks dealing with animal experimentations (ex. EU Directive 2010/63/EU), was identified as the most appropriate developmental stage for detecting xenobiotic-induced metabolomic perturbations. Metabolomic fingerprints were largely chemical- and dose-specific and were reproducible in multiple exposures over a 16-month period; importantly, in the absence of morphological response. Furthermore, chemical-specific responses were detected in the presence of a waste water treatment plant effluent. In addition to improving sensitivity for detecting biological responses to low-level xenobiotic exposures, this approach can provide a powerful means of classifying novel contaminants based on the similarity of the metabolomic response to well-characterized substances, while supplementing functional data generated by OECD TG236 with information on molecular phenotype.

Measuring and Estimating Bioavailability: Linking Exposures to Effects for Improved Ecological Risk Assessment

324 Relating in vitro to in vivo: bioavailability an essential element

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In vitro assays are often applied for unraveling toxicological mechanisms of action. This is major strength of these assays and will also lead to a reduction in animal in vivo testing. Such mechanistic studies are mostly qualitative. To extend the use of these assays to hazard characterization and perform quantitative in vitro to in vivo extrapolations (QIVIVE), the dose in the vitro test should be linked to a dose in a whole animal study. The freely dissolved concentration is one example of a dose metric that may link in vitro (cell medium) to in vivo (blood). In order to be successful, one needs accurate information about the dose in the in vitro test.

Nominal concentrations may be appropriate for "simple compounds", but for more hydrophobic or volatile chemicals, information about the actual dose is needed. Exposure modeling is very useful in identifying these more difficult compounds and can also be applied to estimate the actual bioavailable dose. This presentation will focus on the strengths, but also some of the limitations, of the concept of the freely dissolved concentration. Examples from earlier publications as well as new experimental data for cationic surfactants will be presented to identify advantages and potential limitations in estimation and measuring the actual dose and bioavailability in in vitro studies. New data on the dose of a series of benzalkonium chlorides in a cell assay with a fish gill cell line for example show that deviations of measured concentrations from nominal concentrations in the test medium depend, as expected, on the carbon chain length. However, additional factors that influence these deviations are the exposure time as well as the exposure concentration. Earlier studies have also shown that the technique applied to spike a compound in the medium may affect the outcome of the test. Other dosing systems, based on passive dosing from a sink, may circumvent these confounding factors but such dosing systems are available for only specific types of compounds and it is not feasible to apply those techniques in high throughput screening. Also the hypothesis that the freely dissolved concentration represents the bioavailable and active form is not always correct, in particular in non-equilibrium situations, and this will be illustrated with a few examples. More general strengths, limitations and research recommendations of in vitro-in vivo extrapolations will be discussed.

325 Demonstrating the value of applying generic modeling tools for simulating the behavior of organic chemicals in in vitro test systems

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A large number of in vitro assays and toxicity tests have been developed over the past few decades to complement in vivo assays and tests and to increase our knowledge about potential modes of toxic action. In vitro toxicity testing has been gaining particular prominence over the past decade due to the increased desire to reduce animal testing and the development of large-scale in vitro testing programs (e.g., ToxCast™). In many cases, dose-response relationships in the test are based on nominal (total) concentrations and there is no confirmation of the actual dose corresponding to the response (or lack of response). Concerns over the use of nominal (total) concentrations rather than measured and/or freely-dissolved concentrations in in vitrotesting have been discussed in the literature. Nevertheless, nominal concentrations are frequently the only metrics reported for in vitro tests, especially for high-throughput screening (HTS) applications where conducting measurements is technically challenging, if not impossible. Various publications have presented modeling approaches for simulating the behavior of organic chemicals in in vitro test systems but in general their use is not widespread. The main objective of this presentation is to demonstrate the value of applying generic modeling tools when designing and interpreting in vitro toxicity testing. Two modeling approaches are discussed, i) equilibrium partitioning and ii) toxicokinetic (dynamic), with model output focused on differences between nominal (total) concentrations and metrics more directly relevant for toxicity (freely-dissolved concentrations and cellular concentrations). Using realistic examples, the model applications illustrate how differences between nominal (total) concentrations and freely-dissolved concentrations are influenced by physical-chemical properties (e.g., hydrophobicity) and test conditions (e.g., cell-free vs. cell-based, presence/absence of serum). The modeling tools can be used i) prior to initiating studies to identify testing scenarios that are more or less problematic and ii) to help interpret in vitro toxicity data reported only using nominal (total) concentrations.

326 Transferring in vivo exposure into in vitro assays using silicone to assess the endocrine activity of POPs accumulated in human breast implants

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Persistent organic pollutants (POPs) accumulated in human tissues may pose a risk for human health by interfering with the endocrine system. A new partitioning-controlled dosing approach from silicone was applied in the in vitro H295R steroidogenesis assay to test whether POPs in a mixture composition and at concentrations as found in human silicone implants can interfere with steroidogenesis. Silicone served here as donor of a mixture of POPs while it also acted as sorptive sink for lipophilic hormones produced by the cells. The new dosing method from silicone was compared to conventional solvent-dosing. A mixture of PCBs, PBDEs, HCB and DDT as identified in human silicone breast implants was tested by spiking it into 24-well plate adopted silicone disks. When dosed from silicone, the chemical mixture increased the production of progestagens and androgens in the in vitro assay. However, no changes were observed when co-solvent dosing was applied. Responses were observed in the femtomolar freely dissolved concentration range, which is right in the range of circulating POP levels in humans. The new silicone-based dosing approach allowed (1) linking in vivo exposure levels to in vitro exposure concentrations and (2) linking actual human POP levels to an altered hormone production in a human adrenal cell line in vitro.

327 Free and cellular effect concentrations in high-throughput cell-based bioassays: measurement and models

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In vitro cell-based bioassays could be cost-efficient and ethical alternatives to animal testing in chemical risk assessment. Reasons that prevent in vitro assays from replacing in-vivo tests include difficulties associated with evaluating chemical bioavailability in these systems and ensuring controlled and constant concentrations of chemicals in the cells. In cell assays, bioavailability and biologically effective dose are generally not known and are difficult to quantify due to the small volumes used in these microtiter plate assays. Especially hydrophobic organic chemicals may appear less potent than they really are because their bioavailability is reduced by sorption to protein and lipid components of the growth medium and vials. The cellular concentrations provide a better dose metric for in vitro bioassays and are the point of departure for in vitro to in vivo extrapolations because the effective target concentrations are in principle the same across diverse cell types, and independent whether tested in vivo or in vitro. Here we present a mass balance modeling approach for the assessment of internal exposure in cell-based bioassays that relies on experimentally determined partition coefficients between cells and medium, as well as medium components and water. The partition coefficients in these colloid suspensions were measured with a polymer-desorption method using silicone as third phase. Further model input parameters include the experimentally determined lipid and protein contents of CAFLUX cells and medium. We applied the model to polychlorinated dibenzodioxins in the CAFLUX (Chemically Activated Fluorescent Expression) assay. The CAFLUX uses recombinant mouse hepatoma (H4IIe) cells containing a stably transfected AhR-inducible green florescent protein reporter plasmid. Induction of green fluorescence in these cells occurs in a chemical-, receptor-, dose- and time-specific manner and is a direct measure of the binding/activation of the AhR by chemicals added to the cells. The effect concentrations in this assay were strongly dependent on the medium composition but the modelled cellular concentrations were constant and independent on the medium. After successful experimental validation, the mass balance model was then expanded to ionisable organic chemicals and applied to effect data from the USEPA ToxCast database on the example of GeneBLAzer bioassays, which are reporter gene assays for various hormone receptors and transcription factors.

328 The Aggregate Exposure Pathway (AEP): A conceptual framework for advancing exposure science research and transforming risk assessment

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Recent advances in emerging technologies have enabled new streams of exposure and toxicity data to be generated at higher volumes and speed. These new data offer opportunities to better characterize the intricate interactions among chemical and non-chemical stressors, the environment, and biology in the real world. For example, untargeted analysis of environmental samples can be used to identify mixtures of chemicals commonly found in the same media; and high throughput in vitro assays can be used to identify multiple stressors that are able to interact with the same molecular target to perturb a common biological pathway. However, these opportunities to elucidate the complex relationship between exposures to multiple stressors and multiple human health and ecosystem outcomes can only be realized through more efficient organization of data and knowledge emerging from new technologies and approaches. In toxicological science, the adverse outcome pathway (AOP) framework was conceptualized to organize knowledge of biologically plausible and empirically supported links between perturbations on a biological system at the molecular level and the resulting adverse outcome(s). In exposure science, a similar framework, the aggregate exposure pathway (AEP), is proposed to organize exposure data and predictions. We envision an AEP extending from a stressor's introduction at the source(s), to its fate and transport through environmental media, patterns of external exposure, biokinetic processes, and finally ending at its target site exposure(s). The AEP framework deliberately includes internal dosimetry to allow for integration among AEP, AOP, and dose-response data. In this presentation, the AEP concept will be introduced along with its potential applications, such as identifying primary exposure sources, informing toxicity testing strategies, and improving risk assessment. Disclaimer: Although this work was reviewed by EPA and approved for publication, it may not necessarily reflect official Agency policy.

329 Addressing the challenge of Exposure Science in the 21st Century: A strategy for developing more robust exposure assessment tools

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Risk assessment is generally divided between human health risk assessment (HRA) and environmental risk assessment (ERA), whereby scientific research, policy decisions, and regulatory instruments for HRA and ERA are addressed by individuals with different sets of expertise and knowledge, oftentimes at different institutes, research organizations, and government agencies. Integration of HRA and ERA, however, could provide substantial benefits, particularly by providing a more efficient framework that addresses emerging challenges and issues that have the potential to impact both the environment and human society. For instance, a continuing challenge in chemical risk assessment, associated with the wide use of chemicals in our modern society, regards the lack of relatively quick, inexpensive, and reliable tools that integrate the various aspects of exposure relative to the intrinsic toxicity that an individual chemical may possess. Recently, a framework for enhancing our mechanistic understanding of the processes that influence exposure to chemicals by individuals and environmental systems, referred to as Aggregate Exposure Pathways (AEPs), has been proposed, and which promises to provide a complementary framework to the development of Adverse Outcome Pathways (AOPs). An important difference between an AEP and an AOP, however, is that while AOPs are non-chemical specific, addressing the specific properties of chemicals is key to quantifying exposure, and therefore AEPs must include chemical-specific information. Here we present the development of various tools aimed at better quantifying exposure for use in risk assessment, and which utilizes cross-domain expertise, from both HRA and ERA, to develop an AEP aligned to the use of chemicals used in personal care products. A major emphasis of the strategy described herein targets the development of tools that both reduce and accurately quantify uncertainty associated with assessing consumer exposure to chemical ingredients used in personal care products.

330 Evaluating the Inhalation Bioavailability of Hydrophobic Organic Contaminants in Dust using Isotope Dilution Method

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In current risk assessment, 100% bioavailability is often assumed when evaluating human exposure to hydrophobic organic contaminants (HOCs) in contaminated land. While this has advantage of being precautionary, it may significantly overestimate the amount of pollutant absorbed by biota, including humans. Therefore, understanding the bioavailability of HOCs is of great significance for adequate risk evaluations. One of the principal sources of human exposure to HOCs is direct inhalation with dust in the air, however, few research has been proposed to investigate the bioavailability of HOCs in dust. In this study, we extended the isotopic dilution theory to the use of stable isotope labeled reference compounds to determine the bioavailability of HOCs in dust. A large set of HOCs (DDTs, PAHs, PCBs, PBDEs) will be investigated using two fluids (Gamble solution and simulated epithelial lung fluid) on different types of dust. Tenax-aided desorption experiment will be used to validate the performance of isotope dilution method, the same dust samples will be subjected to sequential desorption in which Tenax beads are used to recover the desorbed HOCs to facilitate measurement. And also, cellular uptake of HOCs form dust in Human A549 lung epithelial cells will be considered. This novel method is expected to be especially useful for bioavailability measurement and human risk assessment.

331 Improving Mechanistic Models for PCB Bioaccumulation in Fish Using Passive Sampling of Freely Dissolved Concentrations

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While empirical results are available in the literature on bioavailability reductions after amendment of PCB-contaminated sediment with a strong sorbent like activated carbon (AC) or biochar, there is a lack of quantitative understanding on how reductions in sediment porewater concentrations and reduced uptake at the base of the food chain impact accumulation in fish. In addition, developing more accurate partitioning models that can predict uptake by pelagic organisms, i.e. algae and zooplankton that serve as food to fish, are key to understanding PCB uptake in fish. With a validated model for bioaccumulation, timely assessments of a remedy progress upon engineering intervention like AC amendment can be performed. This study will present the first robust modeling framework that is able to predict uptake in fish after in-situ remediation that alters bioavailability of pollutants in sediments. Passive sampling measurements are linked to PCB uptake by pelagic and benthic feeding fish, as well as PCB accumulation in algae and zooplankton to provide refined models that will lead to more precise predictions of uptake in the aquatic food chain. PCB exposure to fish was conducted using mummichog (Fundulus heteroclitius) and cory catfish (Corydoras aeneus) in laboratory experiments. Porewater, surface water, and fish tissue concentrations were measured in tanks containing either clean sand, PCB-impacted sediment, or PCB-impacted sediment treated in the field with AC (2.5% d.w.). Benthic blackworms (Lumbriculus variegatus) were exposed to untreated or treated sediments prior to the experiment and were used as food for the fish in the untreated and treated tanks, respectively. AC treatment resulted in 97% reduction in total PCB concentration in porewater and 91% reduction in overlying water. After 90 days of exposure, both fish showed reduced uptake of PCBs in tanks with sediment treated with AC (34 and 74% for cory catfish and mummichog, respectively). Overall, the kinetic model was reasonably accurate in predicting PCB residue in mummichog and led to more accurate predictions than the equilibrium model. For cory catfish the kinetic model over-predicted uptake for most of the congeners and did not improve significantly over the predictions made by the equilibrium model. Ongoing work is focused on the PCB exposure studies using algae and zooplankton and measuring freely dissolved concentration with passive samplers to derive more accurate BCF-K_{OW} correlations.

Advancing Sustainability in SETAC: Implementing the Berlin Declaration

332 Brundtland, The Berlin Declaration, and Beyond

R.J. McCormick, Bureau of Land Management

As we approach the fourth anniversary of the development of SETAC's Berlin Declaration, it is worth reflecting on whence we came, and what the future might hold for us as a society. I will attempt to trace the sustainability history of human society, from pre-history to post-Brundtland, with a focus on the impending, context-enforced, global sustainability paradigm, and thus some implications for the future of our daily resilience endeavours. Humans often carry with them biases of how soil and water and weather organize patterns on a landscape based on where they most recently came from. For example, even after decades of trying to grow olives in Virginia (USA) and failing, social learning did not occur as the immigrants insisted on imposing a world view gained in one region (Europe) on a new world landscape. Open and active learning is a constant challenge that local and global societies will face as the climate continues to change. Unless we approach these novel situations absent the pre-conceived notions and mental filters of how we think our social – ecological systems work, there are likely to be devastating failures. In tying together the principles of the Declaration with resilience and sustainability science, I will present the ecological concepts behind establishing a beachhead on a new landscape (or under a novel climatic regime) that are key to forming a resilient community, rather than just one that is trying to be less unsustainable. Even with some knowledge of the stock and flow of ecosystem services in a place at a time, if your social and cultural knowledge does not know how to exploit those services, ecesis will not occur, and your society will not persist. As noted in early drafts of the Berlin Declaration, dogmatic adherence to social and cultural memes can have unexpected results. The principles in the document offer individuals and society a resilient path to changing mental models and living more sustainably.

333 Sustainability in the face of the Industrialization of Social-Ecological Systems

W. Hauter, Food and Water Watch

The industrialization of social-ecological systems (SES) has allowed for efficiency and in many cases, increased access to basic needs. However, as with everything, do we understand the tradeoffs? Safety, security and accessibility are needed whether we think about food or about energy. Wenonah Hauter, Founder and Director of Food and Water Watch and author of "Foodopoly" and "Frackopoly" will speak on sustainability with a focus on the US food and energy industrialized systems. Wenonah's schedule is such that she is available on Nov 7, 2016 so if it's possible to schedule this session that day, this would work for her as a speaker.

334 Challenges of Sustainability in the Everglades Ecosystem

L. Gunderson, Emory Univ / Dept of Environmental Sciences

Climate change will challenge the adaptive capacity of the Florida Everglades social-ecological system. The Everglades is a subtropical wetland that has been drastically modified over the past century to achieve multiple societal goals: 1) water supply for millions of people, 2) a multi-billion dollar agriculture enterprise, and 3) an international reserve for conservation of biodiversity. Water supply and flood control have largely been successful and, over time, have adapted to seen and unforeseen hydrologic variation. These ecosystem services are sustained by a relatively stable climate. Current agricultural practices are likely unsustainable, as the organic soils that accumulated over thousands of years have been largely oxidized over the past century. For the past two decades, millions of dollars have been spent to recover degraded ecosystem attributes whose resilience was eroded by management efforts. Even with large fiscal resources, recent assessments suggest that the current system is quite limited in it's capacity to experiment and adapt to a changing climate.

335 The Role of Environmental Education in Psychosocial Resilience

C. Stahl, USEPA Region III; B. Duncan, USEPA Region X

In statutory and regulatory contexts, psychosocial effects are not discussed or considered, and yet, when people are asked, these effects are often the most important problem raised. We think of resilience as the capability to "bounce back" when confronted with pollution and disruption. What does resilience mean when applied to communities stressed by anthropogenic activities? One aspect that is oft ignored is psychosocial resilience. What does resilience mean when unwilling stakeholders need to be engaged if the problem is to be addressed? In an experiment conducted during a 2016 global online environmental education course offered by Cornell University, we crowdsourced the 2000+ student participants to ask their ideas about whether and how environmental education could have a role in nurturing psychosocial resilience. Although we initiated the discussion using the mountaintop removal coal mining (MTM) operations as an example of anthropogenic activities having potential psychosocial effects in communities, we asked the global online students to think more broadly to other kinds of activities with similar psychosocial effects. We learned that a current commonly-held conceptual model for environmental education was ill-equipped to help stakeholders and communities address psychosocial resilience and so, an alternative is proposed.

336 Innovations in Resilience – Florida's New Future Legacy

S. Bronson, Florida Earth Foundation

Florida is one of the most diverse states in the US with epic population growth, diminishing natural resources and a conservative political climate. However, there are several dynamic projects and programs that will lead the nation and beyond in innovative efforts to not only retrofit the state for resilience, but create new examples of sustainable systems for both the built and natural environments. Three examples will be discussed of Babcock Ranch, The C-51 project and the Dispersed Water Program that intersect cities and ecological systems in ways that create new paradigms of sustainability.

337 A Required Paradigm Shift to Inform Sustainability

L. Kapustka, LK Consultancy

Increasing numbers of humans and rising expectations for billions to realize improvements in their quality of life are threatening to undermine aspirations for sustainability. These pressures are mounting in ways that appear likely to disrupt stability and predictability of social-ecological systems. For more than two decades, we have known that the traditional approaches of reductionist science are insufficient in terms of understanding and managing outcomes of either community wellbeing or ecological processes that deliver ecosystem services. Reductionist approaches are embedded in a collective mentality of western societies and expressed in laws and policies that attempt to reduce the complexity of societies and ecosystems to linear cause-effect chains. Sustainability Science, which emerged from Systems Ecology and calls for a Post-modern Science, is based on the recognition that societies and ecologies are fundamentally non-linear in their behaviour due in large part to multiple nested feedback loops (some reinforcing or positive; some dampening or negative) that operate on different spatial and temporal scales. There is also the recognition that the welfare of societies is ultimately dependent on reasonably constant flows of ecological goods and services. Despite a general acknowledgement of these aspects of the social-ecological systems, our political system and our science remain anchored in short-term, dramatic responses and demonstrable causal chains to explain effects. Resilience of social-ecological systems is defined by biodiversity/redundancy, connectivity, and slow variables and feedbacks operating in complex adaptive systems. From the societal perspective, managing resilience requires learning and experimentation, broad stakeholder participation, and polycentric governance systems. Rather than a clean causal chain, these systems tend to respond along a number of trajectories (i.e., one-to-many pathways). Moreover, the determinative pressures are more likely to be the slow variables that more often than not are ignored in reductionist approaches as being insignificant. These concepts will be explored by a holistic examination of the capacity of agro-ecosystems to provide sustained flows of multiple ecosystem services. Attention will be drawn to the short-comings of the dominant reductionist approach that focuses primarily on short-term yield.

Lab Data, Hazard, Risk and Regulation of Endocrine Active Chemicals – So What? The Big Picture from Little Pieces

338 Lessons from history – how good are we at predicting the environmental risks of endocrine disrupters?

P. Matthiessen, Unaffiliated

A recent SETAC Pellston Workshop™ 'Environmental hazard and risk assessment approaches for endocrine-active substances – EHRA' came to the unanimous conclusion that providing environmental exposure, effects on relevant taxa/life-stages, delayed effects and dose/concentration-response relationships are adequately characterized, then conducting environmental risk assessment of endocrine disrupting substances (EDSs) is scientifically sound. This was a victory for science and common sense, and united both sides of an increasingly sterile debate about hazard vs risk assessment. However, before we get completely carried away with our own good judgement, it is worth looking at our success rate in predicting and avoiding environmental impacts from EDSs. Some notorious examples which caused and are still causing serious damage, including tributyltin, DDT and some PCBs, entered the market long before environmental evaluations became routine, but we can still ask if modern risk assessment procedures would have detected them (yes and no). Others, such as ethynylestradiol and diclofenac, caused serious impacts which should have been predicted but were not. Yet others, such as the azole fungicides, have been identified as EDSs and may become the subject of bans in certain regions, but there is no field evidence that they are causing harm. The paper will survey this mixed bag of examples and attempt to draw one or two lessons. One conclusion is that, although environmental risk assessment is nowadays a reasonably robust procedure (even for EDSs!), it must go hand in hand with good quality environmental monitoring. This is because no system of chemicals regulation can ever be made perfect without preventing all chemicals from entering the market. Environmental monitoring can and should act as the 'fielder' for catching stray balls. An example of completely unexpected impacts of sewage effluents on the hormonal stress response in fish will be presented as a cautionary tale.

339 Examining the disconnect between laboratory studies of endocrine active compounds and perceived ecological consequences

H.I. Schoenfuss, St. Cloud State Univ / Aquatic Toxicology Laboratory

Laboratory exposure studies of endocrine active compounds, including xenoestrogens and several classes of prescription pharmaceuticals have provided consistent evidence for their adverse effects on exposed organisms. At the same time, field studies have struggled to replicate laboratory findings or have documented effects not observed in the laboratory. This presentation will provide multiple lines of quantitative evidence to argue that the mode-of-action of many endocrine active compounds, coupled with the often overlooked biological complexity of aquatic ecosystems, may explain this disconnect. For example, constant environmental conditions, required in the laboratory to exclude confounding factors, mask the overriding physiological impact of physic-chemical conditions on resident wildlife in field studies. Furthermore, the limitations on within-species and across-species interactions in the laboratory exclude exacerbating circumstances faced by organisms exposed in environmental settings. Finally, fluctuations of mixture composition and concentrations commonly observed in aquatic ecosystems, modulate organismal responses taken for granted in laboratory exposure studies. Although adding complexity, these obstacles to linking laboratory results to field observations should be viewed as an opportunity to expand or understanding of biological complexity rather then a limiting factor in our ability to predict the environmental effects of endocrine active compounds.

340 Social hierarchy modulates biomarker expression in fish exposed to contaminants of emerging concern

J. Ivanova, St Cloud State Univ / Biology; H.I. Schoenfuss, St. Cloud State Univ / Aquatic Toxicology Laboratory; R. Wang, USEPA / Ecological Exposure Research Division

Male fathead minnows (Pimephales promelas) in single-sex exposure groups establish social hierarchy as is evident by the differential expression of secondary sex characteristics (SSCs). SSCs expression is linked to circulating androgens concentrations, which are under the control of hypothalamo-pituitary-gonadal (HPG) axis. We tested the hypothesis that dominant and subordinate males belong to two biologically different subpopulations by pooling ten previously conducted studies in a Meta analysis. All experiments utilized the fish of same age from the same breeding facility and exposed at comparable environmental conditions. The results indicate that control males separated into two distinct subpopulations by SSC score (MANOVA, $p < 0.0001$), and that they also expressed different vitellogenin (VTG) concentrations. Males exposed to both E1 and E2 also formed two subpopulations relative to SSC expression ($p < 0.0001$ and $p < 0.0264$, respectively) but showed higher VTG concentrations when compared to controls. The greatest separation among the subgroups was found in males exposed to mood-altering pharmaceuticals ($p < 0.0001$) while having VTG concentrations comparable to controls. Our results suggest that male population of fathead minnows is heterogeneous. Our results also indicate that social hierarchy influences the fish responses to the contaminants of emerging concern (CECs).

341 Problem Formulation for Hazard Identification of EACs Requires Potency

C. Borgert, Applied Pharmacology & Toxicology, Inc.

Some argue that Hazard Identification (HI) for EAC's should be based on the potential for a chemical to act via an endocrine mode of action (MoA) rather than on the demonstration of adverse effects produced via an endocrine MoA. Thus, cellular or molecular assays would be given prominence in HI for EACs similar to the use of mutagenicity assays in the HI for genotoxicity. But, fundamental differences in these MoAs dictate a different problem formulation step for HI of EACs, where the critical question is not whether a molecular or cellular response is elicited, but with what potency. Vital signaling functions of the endocrine system require it to continuously discriminate the biological information conveyed by potent endogenous hormones from a more concentrated background of structurally similar, endogenous molecules with low hormonal potential. This obligatory ability to discriminate important hormonal signals from background noise is achieved through differential potency and laws of mass action, which together determine receptor occupancy and activation state in target cells. Discrimination based on potency can be theoretically-derived and corroborated by experimentally and clinically observable potency thresholds, without which normal physiological functions would be impossible. Although it has been argued that because the endocrine system is basally activated by endogenous hormones, very small amounts of low-potency chemicals could alter its function, simple receptor occupancy calculations reveal that in contrast, trillions of molecules would be required to change receptor occupancy by any measurable degree. The requirement for a sufficient change in receptor occupancy and cellular activation state, both of which depend on potency and mass action, forms the theoretical basis for potency thresholds derived directly from established principles of endocrine pharmacology. The incorporation of potency thresholds into HI is illustrated here using endogenous estrogens, androgens, and essential fatty acids as examples. The argument that thresholds cannot be proved empirically is mathematically correct, but practically irrelevant for safety assessment. Instead, the relevant question is the dose required to produce a specified level of effect that is deemed to be adverse. Conclusion: Without sufficient potency, there is no hazard, but there is a risk of fictitious endocrine disruption.

342 Missing links in our understanding of estrogenic compounds; chemical quantitation vs. biological assessment – where do we go from here?

N.W. Shappell, USDA-ARS / Biosciences Research Lab

The literature has become replete with reports quantifying estrogenic chemicals in the environment ranging from natural hormones to plasticizers. In addition, laboratories have developed in vitro assays to assess estrogenic activity of both environmental samples and pure chemicals. Information pertaining to the in vivo effects of the chemicals is much less exhaustive and the correlation to in vitro results is often ignored. Estrogenic potency of chemicals can vary significantly depending on the source of transfected estrogen receptor. One lab found trout versus human receptors were 62 fold more sensitive to one compound, while for another compound trout receptors were only 0.04% as responsive as human receptors. The same lab found the in vivo response to a chemical was 1/10,000th as estrogenic as estradiol, while by in vitro assay (transfected estradiol receptors from fish) the chemical was 1/600th as estrogenic. Chemical additivity has been reported from both in vitro and in vivo laboratory exposures, though similar additivity has not been reported for humans “dosed” with multiple estrogenic chemicals. While agriculture and wastewater treatment plants have been implicated as point source contributors of estrogenic contaminants in surface waters, studies of many water bodies have failed to detect these contaminants at biologically relevant concentrations. Further consideration is needed relative to the specific conditions of area of release relative to the impact of various estrogenic agricultural inputs. Surface water release of these compounds in arid regions could result in significant environmental impacts, while posing no problem in less arid regions. Unfortunately, several recent reports have resorted to quantifying estrogen concentration as a sum of all estrogens, ignoring relative estrogenic potential of the specific estrogen. A simple modification of concentration vs. prevalence data would allow for improved risk assessment. The relevance of these findings will be discussed and suggestions for future research directions made.

343 Challenges in ecotoxicology – what does controlled and standardized results mean in a complex and dynamic environment?

T. Brodin, Umeå Univ / Dept of Ecology and Environmental Science; J. Fick, Umeå Univ / Dept of Chemistry

Pharmaceutical residues are found in many aquatic ecosystems all over the world. Groups of pharmaceuticals of particular interest for potential ecological effects are EDC:s (endocrine disruption compounds) and anxiolytic drugs since they are designed to alter human physiology and/or behavior. There is a growing awareness among ecologists that behavioral variation and alterations are important for individual performance, ecosystem function, and species evolution. In ecotoxicology it has been recognised that such behavioral alterations may be caused by contaminants found in natural systems. Recent studies have shown that both EDC:s and anxiolytic drugs can be found present in environmental concentrations high enough to effect behavior of fish. Very little is, however, known about how/if these compounds affect the behavior of other aquatic organisms, or how they interact with natural stressors. In a recent meta-study, comparing studies using behavioral endpoints to studies with acute lethality, development, or reproduction as endpoints, it was concluded that behavioral studies warrant further attention as tools for assessing the effects of environmental contaminants. Information of how/if other key species are affected by exposure is crucial to be able to predict to what extent major ecological effects can be expected. Higher risk for severe ecological impact is predicted if the degree of which species specific effects occurs. That is, if pharmaceutical contamination induce effects that are asymmetric between species or even between life-stages within species. Such asymmetric effects can lead to shifts in species densities and compositions through altered competition and predator-prey interactions. The ecological ramifications of such pharmaceutically induced asymmetric behavioral changes are still unclear, as to date no studies have investigated the issue. Here, I will present empirical evidence for asymmetric behavioral effects of pharmaceutical exposure both within and between trophic levels and discuss potential ecological consequences thereof. I will also illustrate, using novel data, the importance of including natural stressors (e.g. predation risk) for the outcome of ecotoxicological testing of pharmaceutical. Finally I will discuss the problems we face when trying to extrapolate results found in the lab to what will happen in the wild and suggest one possible way of bridging the gap between lab and field.

Assessing Ecological Risk to Inland Environments Due to Increases in Major Ions

344 What we know and don’t know about major ion pollution from 15 years of research in Australia

B.J. Kefford, Univ of Canberra / Inst for Applied Ecology

There has been much concern in Australia about ecological effects of increased salinity, or major ions, from rising saline groundwater which initiated a series of research project starting in about 2000 into the impact of rising salinity on freshwater organisms, community and ecosystems. This research showed certain taxa, e.g. Ephemeroptera (mayflies), are particularly sensitive to salinity rises while others, e.g. macro-Crustaceans, tend to be tolerant. Some, but not all, freshwater taxa actually do somewhat better sub-lethally at slightly elevated salinity. There is, however, a contradiction between the results of field and laboratory studies, in that risk assessment based on single species toxicity tests suggested that no species would be at risk at 1 mS/cm, while at that salinity there is a 50% loss of regional Ephemeroptera, Plecoptera and Trichoptera species richness. Recently attention has shifted to concern about saline effluents from coal mines and gas extracted from coal bed, or coal bed saline effluents. There are many similarities between salinity increases from rising saline groundwater and those from resource extraction but also an important difference. The former saline waters are NaCl dominated and tend to have ionic proportions similar to sea water. In contrast the ionic proportions in waters from coal beds are much more varied with relatively higher proportions of bicarbonates and sulphates occurring. Variations in ionic proportions both change the toxicity and the relative sensitivity of different taxa, producing a considerable challenge. Significant progress may not occur until the physiological mechanisms underlying this variation are understood.

345 Laboratory Evaluations on the Effects of Dissolved Solids on Benthic Macroinvertebrate Communities and Individual Taxa from West Virginia Streams

M.Y. Armstead, M. Wilson, Marshall Univ / Integrated Science and Technology

Much attention has focused on declining macroinvertebrate communities in the Appalachian region as a result of increased dissolved solids from mining activities. Evidence for levels of dissolved solids impairing communities has been primarily based on correlation of large field surveys with landscape disturbance area and water quality from monitoring programs. Field studies specifically evaluating the effects of dissolved solids on native taxa have increased recently, but laboratory confirmation of field surveys is sparse. Hindrances to laboratory confirmation of field surveys include difficulty collecting and handling native taxa, temporal and spatial variability in condition of organisms collected for testing, and difficulty procuring sensitive life stages. Studies on the effects of dissolved solids, measured as specific conductance, on macroinvertebrates from streams in the mining region of West Virginia have been ongoing in our laboratory for 5 years. Evaluations have included: mesocosm studies of field-collected macroinvertebrates exposed to a simulated mine discharge with conductivity ranging from 100 µS/cm to 2400 µS/cm, a multivariate evaluation of the effect of conductivity on individual taxa encountered in the mesocosm studies; and recently, toxicity bioassays with laboratory reared larval mayflies. Results of the multiple projects indicate variable community level effects with initial taxa composition significant in overall test significance despite similarities in community metrics at test initiation. Individual taxa in both Ephemeroptera and Trichoptera orders demonstrate similar sensitivity to increased dissolved solids. Implications of bioassay results from tests utilizing laboratory reared larval mayflies are preliminary as salts are being used to demonstrate fitness of our cultured organisms. However, the relative responses of multiple taxa and comparison of newly hatched and older individuals is informative. Results of the individual projects and trends in our findings will be presented with a discussion of implications for managing current conditions in the mining regions of West Virginia.

346 Individual and community responses in stream mesocosms with different ionic compositions of conductivity and compared to a field-based benchmark

C.T. Nietch, USEPA / Water Supply and Water Resources Division; J.M. Lazorchak, USEPA / Office of Research and Development; D.R. Mount, USEPA / ORD NHEERL Mid-Continent Ecology Division Duluth; W.H. Clements, Colorado State Univ / Dept of Fish Wildlife and Conservation Biology

Several anthropogenic activities cause excess total dissolved solids (TDS) content and its correlate, specific conductivity, in surface waters due to increases in the major geochemical ions (e.g., Na, Ca, Cl, SO_4). However, the relative concentrations of major ions varies with the source, and single-species tests have revealed that ion compositional differences can affect their toxicity. Within the central Appalachian region of the U.S., oil and gas production and surface coal extraction activities are two sources of excess TDS for surface waters; releases from the former are typically dominated by Na, Ca, and Cl, and the latter by Ca, Mg, SO_4, and HCO_3. A stream mesocosm study evaluated the effects of excess TDS from both discharge types against a natural background chemistry by dosing two recipes mimicking each type of excess TDS over a 56d period. Hazard concentrations (HCs) for specific conductivity from EC50 response sensitivity distributions derived from the mesocosm data differed, with HC5 estimates of 792 µS/cm (685 to 933, 95% CI) for the simulated oil and gas produced water excess TDS, and 434 µS/cm (338 to 584, 95% CI) for the recipe simulating streams receiving surface coal mining discharges. While the mesocosm study was originally intended to evaluate TDS limits in NPDES permits, it also serves a dual purpose to evaluate the field based conductivity benchmark of Cormier et al. The benchmark, circa 300 µS/cm, was derived from analyses of benthic samples taken from central Appalachian streams, and was meant to protect communities exposed to ion mixtures comparable to the ionic composition of the surface coal mining recipe dosed to the mesocosms. As the lower 95% confidence interval of the HC5 estimate for the coal mining recipe was 338 µS/cm, these results support the field benchmark as protective for the specific conditions for which it was developed. In keeping with the applicability restrictions imposed by Cormier et al however, the higher HC5 value from the mesocosms exposed to the oil and gas produced water recipe highlights how important it is to keep the context for the benchmark in mind when evaluating risks of discharges with different ionic compositions or for different receiving waters.

347 What Physiological Research on Ion Transport Suggests about the Relationship between Anion Toxicity and Hardness

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Bioassay studies have purported to measure the toxicity of anions, such as Cl^- and SO_4^{2-}, in freshwater animals and in several cases have related this toxicity to hardness. However, considering the ion transport physiology of freshwater fish, crustaceans, aquatic insects, and molluscs, attributing the observed toxicity to the anions alone may be questionable, as is the apparent relationship with hardness. Cl^- is known to be transported across apical epithelial membranes of animals from freshwater by a HCO_3^-/Cl^--exchanger, also known as an anion exchanger. Epithelial SO_4^{2-} transporters are not currently known, and SO_4^{2-} is considered relatively impermeant to external epithelial membranes. Hardness is known to decrease the toxicity of divalent metals, because metal ions and Ca^{2+} compete for Ca^{2+} transporters. Consequently, increased Ca^{2+} inhibits metal uptake. In these bioassays, Na^+ salts have generally been used to increase anion concentrations, so Na^+ is increased in addition to the anion but not other ions. In Crustacea, and probably Mollusca, there is mutual competitive inhibition of uptake between Na^+ and Ca^{2+} at an additional transporter, an electrogenic $2Na^+$ or Ca^{2+}/H^+-exchanger, suggesting the observed relationship is associated with Na^+ and hardness. No similar mechanism can explain a hypothesized relationship between hardness and anion toxicity. The views expressed are those of the author and do not necessarily reflect the views or policies of the USEPA.

348 Calcite production is lethal to a model freshwater invertebrate

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Salinization of freshwaters can, aside from increasing the total ion content in water, result in extensive deposition of calcite in freshwater ecosystems. While extensive calcite deposition occurs naturally in a few aquatic ecosystems, the organisms inhabiting these ecosystems are typically saline tolerant. In this study, we determined whether a model freshwater organism, Daphnia magna, is negatively affected by calcite production or by the components required for calcite production (i.e. high alkalinity and water hardness). Results from a series of 48 hr – 96 hr toxicity experiments revealed that rapid shifts in alkalinity and water hardness were not lethal if no calcite precipitated. However, if water conditions supported calcite precipitation, ≥98 % of D. magna neonates died within 96 hrs of exposure. To determine the potential ecological relevance of our tests, we collected calcite from a mining-affected stream and examined it for the presence of entrapped organisms. Organisms were found embedded in this field collected calcite suggesting that species loss may also be occurring in streams affected by resource extraction where extensive calcite precipitation is induced. Our results support the development of a water hardness + alkalinity guideline for the protection of aquatic life.

349 Salt and Mayflies – chronic exposure and time independence

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Saline effluent discharges have been a growing concern over the recent years. Suggested safe salinity levels from risk assessments based on species sensitivity distributions (SSDs) suggest no adverse effects at about 1 mS/cm but at this salinity about 50% of the Ephmeeroptera, Plecoptera and Thricoptera (EPT) species are lost in Australia and similar losses occur elsewhere. As shown by Guillaume et al (2015), current SSD methods deriving protection values lack ecological realism and statistical soundness. In particular SSDs are unable to account for time-dependence issues and therefore is unable to truly provide safe protection targets for salinity. Guillaume et al 2015 suggest the use of no effect concentrations (NEC) which are theoretically time independent. But there is a lack of empirical data showing that they are in fact time independently.

Furthermore mortality from salt sensitive EPT species from chronic exposure able to explain the loss of protection at low salinity levels. We conducted experiments with 5 species of EPT collected from a reference site with no salinity exposure and measured mortality over 15- 39 days depending on the species. Results show that NEC values calculated over 96 hours of exposure were very similar to NEC over exposures of weeks, suggesting that the NEC is really time independent. NEC values for EPT were, however, still higher than salinity in which EPT species are lost in nature, suggesting that sub-lethal and/or indirect effects need to be investigated. Such investigations are currently underway.

350 The confounding effects of extremely high salt concentrations in assessing impact of hydraulic fracturing mixtures on rainbow trout

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Hydraulic fracturing involves pumping a mixture of water, fracture proppants, and industrial fluid into deep wells at high pressures to allow for the recovery of tight gas and oil reserves. Flow-back and produced wastewaters (FPW) are extremely high in salinity (up to 330,000 ppt), and also contain elevated concentrations of organic and metal contaminants. From 2005-2013, there were >2500 documented flow-back fluid spills in Alberta alone, and of these, 115 were large spills into either flowing water, freshwater lakes or wetlands. Little data exists on the impact of these complex mixtures on native fish species. Using juvenile rainbow trout, 48 hour acute toxicity tests were conducted to replicate spill effects. Fish were exposed to 7.5% of the total FPW, or salt-matched controls. Gill histology showed significant changes in lamellar width, inter-lamellar cell masses, and changes in cell type with exposure to FPW, similar to effects seen in salt-matched controls. FPW- and salt-matched control exposed fish also showed signs of oxidative stress, with decreased levels of SOD with 50% reduction in the highest exposure tested in the gill and liver, and increased catalase activity in the same tissues. The pattern of measured changes represents a unique signature of impact, suggesting an approach that could be used to track exposures, facilitating an understanding of spill impacts and helping to define a zone of biological influence of the spill. Overall, these data point to significant impacts of salt stress as a confounding factor in understanding the effects of FPW spills. These data will aid in the design of post-spill environmental effects monitoring and risk assessment.

351 Relationship between fish species extirpation and specific conductivity

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We investigated the effect of increases in ionic concentration (specific conductivity) on fish species in Central Appalachia. Effect levels were calculated using the extirpation concentration at the 95th centile (XC95) of a weighted cumulative distribution function, a method previously developed using benthic invertebrate genera. As might be predicted mathematically, the genus XC95 values for fish species are higher than those for most of their constituent species. Thus, genus-level effect thresholds may not be predictive of species level effects. However, no fish species, with >25 occurrences had an XC95 less than the benthic invertebrate field-based benchmark in Central Appalachia. Etheostoma baileyi, the emerald darter, had a predicted XC95 value of 322 µS/cm. Thus, a genus level benchmark at the 5th centile of a benthic invertebrate XC95 distribution is protective of most fish species. However, fish are likely to be affected well before extirpation. Access to species level XC95 values and their exposure response models allows evaluation of the effects of increasing ionic concentration on individual species. For example, the probability of observing brook trout, Salvelinus fontinalis, decreases by 10% at 35 µS/cm, by half at 135 µS/cm, and by 95% (extirpated) at 508 µS/cm. Fish species' responses to increasing specific conductivity may be useful for evaluating the effect of salts and may be diagnostic of impairments caused directly or indirectly by ionoregulatory stress. These are the authors views and do not necessarily represent the views or policies of USEPA.

Novel Mechanisms of Nanomaterial Toxicity Through Direct Exposure or Indirect Interactions with Environmental Components – Part 2

352 Roles of Direct and Indirect Light-Induced Transformations of Carbon Nanomaterials in Exposures in Aquatic Systems

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Carbon nanomaterials (CNMs) such as fullerenes, carbon nanotubes and graphene-based nanomaterials have a variety of useful characteristics such as extraordinary electron and heat conducting abilities, optical absorption and mechanical properties, and potential applications in transistors and polymer nanocomposites. CNMs often are used in consumer and industrial products as composites with other materials such as polymers. The release of CNMs such as multiwalled carbon nanotubes (MWCNT) from the composites is stimulated by weathering induced by light exposure. Release can be followed by using ICP-MS to measure metal residues that are associated with the nanotubes, by UV-visible techniques or by TEM images of particles that can be leached from the weathered surfaces. In most cases the released CNMs are associated with fragments of the polymer matrix. Little is known about the fate of these unusual CNT-containing fragments but recent studies of microplastic residues in lakes and the ocean suggest that they may accumulate in aquatic organisms. Other studies have shown that rapid sunlight absorption by CNMs such as graphene oxide and carboxylated nanotubes leads to rapid conversions to carbon dioxide and persistent, low-molecular weight photoproducts. Indirect pathways also are available for light-induced transformations of CNMs in natural waters. The indirect pathways are driven by reactive transients such as hydroxyl radicals that are produced by photoreactions of natural organic matter (NOM) and inorganic species such as nitrate, metals and peroxides. The objectives of this presentation are to compare both direct and indirect photochemical pathways for selected CNM transformations driven by sunlight exposure, focusing on structural changes and other factors that influence exposure to the CNMs and their photoproducts in natural waters. We will emphasize carbon nanotubes and graphene oxide in the presentation. Another objective is to define relationships and data that can be used to predictively model effects of CNMs on sunlight-induced photoproduction of reactive transients such as excited triplet states and reactive oxygen species that mediate toxic effects of CNMs and their photoproducts.

353 Predicting Hydroxyl Radical Production from Irradiated Titanium Dioxide Nanoparticles Under Varying Conditions

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Organism toxicity to titanium dioxide nanoparticles (TiO_2 NPs) has been quantified as a response to nanoparticle concentration. While this is a satisfactory explanation for most toxicants, TiO_2 nanoparticle toxicity is highly dependent on environmental conditions. TiO_2 NPs produce reactive oxygen species (ROS), primarily hydroxyl radicals, upon exposure to UV light in aqueous solutions. Quantifying hydroxyl radical formation during TiO_2 NP exposure and using that as the independent parameter in dose response relationships may better reflect the toxicological impact. Our previous research has established that TiO_2 NP toxic dose to Daphnia magna decreases by four orders of magnitude, from 1110 mg/L NPs(48h LC_{50}) under cool white fluorescent tubes to 0.202 mg/L NPs(48h LC_{50}) under ultraviolet lights. This increase in toxicity is due to the production of hydroxyl radicals and the subsequent biological interactions of the hydroxyl radical with the organism. The production of hydroxyl radicals can be influenced by multiple environmental factors such as the contribution of NOM to turbidity, radical quenching, particle aggregation, and the intensity of UV light. The goal of this research was to characterize the influence of these external parameters on hydroxyl radical formation. Parameters that were investigated include the following: light intensity and duration, concentration of dissolved organic carbon, and concentration of TiO_2 NPs. Based on measurements from fluorescent spectroscopy, we have built a model that utilizes site-specific water quality and particle specific attributes to better describe the production of hydroxyl radicals generated by TiO_2 NPs. This model will facilitate the development of site-specific water quality criteria for TiO_2 NPs, and is being applied to develop dose-response toxicity curves to D. magna based on hydroxyl concentration.

354 Protein Corona Predicts Biologic Response and Toxicity Mechanisms of Silver Nanoparticles in Fish

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Nanoparticles (NPs) are widely used in daily products, medicine and nanotechnology increasing the likelihood of exposure. Upon exposure, NPs encounter plasma proteins resulting in the formation of a “protein corona” which can modify the biological identity of NPs and affect their distribution and toxicity. In order to better understand the toxic mechanisms of silver nanoparticles (AgNPs) in fish, our research investigated the protein corona from polyvinylpyrrolidone-coated AgNPs (PVP-AgNPs) incubated with fish plasma. Plasma was collected from adult smallmouth bass and incubated with PVP-AgNPs for 1 and 24 h. Nanoparticle tracking analysis (NTA) was used to quantify changes in PVP-AgNP size after incubation. After 1 h of incubation, size increased from 52.6 nm to 57.3 nm and to 56.1 nm in females and males, respectively. After 24 h, PVP-AgNPs continued to increase in size to 60.8 nm and 58.0 nm in females and males, respectively. We then used high-resolution liquid chromatography tandem mass spectrometry (LC-MS/MS) to identify the proteins. We identified proteins associated with the immune system (immunoglobulin and complement proteins), heavy-metal binding proteins, hemoglobin, apolipoproteins, vitellogenin and zona pellucida, the latter two found only in females which could explain the larger size attained in this gender. Interestingly, albumin, an abundant plasma protein, was not found to be part of the corona in these fish. The study of the formation and characterization of protein corona will increase our understanding on the toxic mechanisms of NPs in vivo. This is the first study to examine protein corona in fish plasma.

355 Investigating mitochondrial toxicity of the Metal oxide nanoparticle ZnO

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Metal oxide nanoparticles (NPs) such as ZnO are frequently used in consumer products such as sunscreen, cosmetics, toothpaste, and textiles. It is well established that various toxic effects caused by nanoparticles are associated with oxidative stress. Thereby we asked if mitochondria is a susceptible target. The model organism Caenorhabditis elegans (C. elegans) provides an excellent in vivo model for this study. Previously, our studies have shown that ZnO NPs induced oxidative stress and increased germ cell apoptosis. In this study we firstly measured the expressions of oxidative stress response and mitochondrial function related genes, including drp-1, fzo-1, eat-3, moma-1, immt-1, chch-3, immt-2, dic-1, ced-9, egl-1, fis-1, fis-2, mff-1, mff-2, act-3, nd4, sod-2, and sod-3. Over 2,000 worms were plated onto each of three ZnO NP treatment groups, including control (614µM, 61.4µM, and 0µM). Worms were exposed for approximately 68 hours at 20°C until worms reached the early adult stage. Then RNAs were extracted and subjected to quantitative RT-PCR analysis. In the 61.4µM treatment, regulation was significantly disrupted ($p< 0.05$) compared to control for moma-1, mff-1, and sod-3. Both moma-1 and sod-3 were down-regulated with fold changes of 0.75 and 0.46, respectively; whereas mff-1 was upregulated by a fold of 1.83. In the 614µM treatment, regulation was significantly altered for eat-3, dic-1, fis-1, fis-2, immt-2, act-3, mff-1, mff-2, sod-2, and sod-3 compared to control. The sod-3 was downregulated by a fold of .30, and all other significant genes were upregulated. Upregulated fold changes were as follows: 4.31 for eat-3, 8.08 for dic-1, 1.85 for fis-1, 1.73 for fis-2, 1.44 for immt-2, 2.55 for act-3, 1.25 for mff-1, 1.83 for mff-2, and 1.93 for sod-3. Based on the gene expression results, several mutant strains were selected for testing their sensitivity to ZnO NP exposure.

356 In vivo exposure to gold nanoparticles alters steroidogenesis gene regulation in the liver irrespective of exposure route in Danio rerio

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Gold nanoparticles (nAu) are being developed for applications as a novel drug delivery system allowing for targeted delivery into cells. The dose concentration of in vitro studies is known however with in vivo exposures the delivered dose is often unknown; therefore a range of concentrations were used to determine possible effects on the steriodogenesis pathway. Adult Danio rerio were exposed to nine concentrations of nAu ranging from 5 mg/l to 45 mg/l for 96 h following standard OECD protocols. Pooled liver samples of each concentration were used for Microarray as well as RT-PCR analysis to determine changes in genes relating to the steriodogenesis. The Cytochrome P450 activity (mM/mg protein) in the liver was also determined by using an ELISA method. Using gene ontology biological process showed genes with more than 65% activation included hormone biosynthetic, estrogen metabolic and estrogen biosynthetic process while molecular function genes above 65% activation included those involved in estradiol and steroid dehydrogenase activity. The fold changes compared to control showed different responses between male and female fish, where male fish had a greater response. The Cyp1 genes showed up regulation in male fish while no response was seen in female fish. Cyp11a1 and Cyp17a1 showed up regulation in the concentration range of 20mg/l and 40mg/l in both male and female fish but no change or down regulation was observed at the remaining concentrations. Cyp19a showed up regulation in male fish but slight down regulation in female fish. The P450 kit was showed no significant differences when compared to control but a higher standard deviation was present at concentrations of 25mg/l and above. Characterization of nAu revealed that at 20, 25, 40 and 45 mg/l the surface charge was anionic and further investigation revealed that nAu were present in the gut of the fish at 40 and 45 mg/l due to ingestion of particle agglomerations which sedimented out of solution. A hormetic response was seen in genes associated with steroidogenesis across a range of concentrations and similar genetic responses were activated regardless of uptake method.

357 The impact of graphene oxide and silver nanoparticles on immune pathways

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Nanoparticles (NP) have attracted attention due to their unique properties. Due to the wide range of applications of NPs in products such as pharmaceuticals, energy trapping systems, clothes, and electrical components, concerns are raised over impacts that large scale production and use of NPs may have on human health and the environment. Although data is available on the acute toxicity of several types of NPs, very little is known about their impacts on physiological systems and biological pathways. The current study investigated the effects of graphene oxide and silver nanoparticles NPs on immune pathways. Commercially available silver nanoparticles and in-house prepared graphene oxide particles were used. Particles were partially characterized using physicochemical techniques to determine their composition, surface charge and diameter. Three cell culture systems were used namely human whole blood primary cultures, B-lymphocytes and RAW264.7 macrophages. Cells were incubated with nanoparticles in the presence and absence of mitogens (lipopolysaccharide or phytohemagglutinin). At the end of the incubation period the cells were screened for cell viability using the WST1 assay system and cytokines representative of inflammation (IL-6), humoral immunity (IL-10) and cell mediated immunity (IFN gamma) using ELISA. Supernatants of B-cells were screened for antibody synthesis using an ELISA. Raw264.7 cells were analysed for nitric oxide, heat shock protein, nitric oxide synthase and cyclooxygenase using Western Blotting. Cell viability assays show that silver NPs have no impact on cytotoxicity and nitric oxide, IL-10, IFN gamma and antibody synthesis. However, silver NPs stimulated IL-6 production by lipopolysaccharide activated whole blood cultures. Silver NPs increased the production of heat shock protein 70, but inhibited the production of nitric oxide synthase and cyclooxygenase in stimulated RAW cell macrophages. Studies using graphene oxide nanoparticles indicate that although these particles are not cytotoxic, they activate IL-6 and nitric oxide synthesis of lipopolysaccharide stimulated RAW264.7 macrophages. In conclusion, data generated shows that silver and graphene oxide nanoparticles are not cytotoxic. However, some results generated indicate that both nanoparticles may cause inflammation upon exposure.

358 Impact of silver nanowire length, diameter, and surface chemistry on rainbow trout RTgillW1 and RTgutGC cell lines

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Silver nanowires (AgNWs) are rapidly displacing the current technology for touchscreen displays, and additional AgNW applications are becoming available in the form of solar cells and conformational electronics like OLED displays. Despite a push to understand the impacts of engineered nanomaterials on human and environmental health, there is a lack of knowledge regarding AgNW toxicity to biological systems. This project used RTgillW1 and RTgutGC cell lines to probe specific characteristics of AgNWs that make them more or less toxic to organisms. AgNWs were synthesized with a variety of lengths, diameters, and surface chemistries. Cells were seeded at a density of 4,000 cells per well in 384 well plates and exposed to AgNWs (0-25 µg/mL) for different periods of time (4, 12, 24, and 48 hours). $AgNO_3$ was used as a positive control. Cytotoxicity was measured by nuclear morphology, plasma membrane and cytoskeleton integrity, mitochondrial membrane potential, H2AX DNA damage, ROS generation, lysosome pH and mass, and apoptosis. Changes to bioenergetics parameters (glycolysis, aerobic respiration, mitochondrial function) were also measured. Results indicate that AgNWs exhibit altered patterns of transport, cellular uptake, and asbestos-like toxic injury due to the high aspect ratio of the nanowires. Both short and long nanowires were internalized by the cells and induced cytotoxicity; however, long nanowires generated slightly higher levels of ROS and apoptosis-related events compared to short nanowires. Surface chemistry and media conditions were also found to influence toxicity. Future work will probe for toxic effects of AgNWs to rainbow trout larvae so that endpoints between in vitro and in vivo toxicity can be compared. These results will help scientists and engineers synthesize silver nanowires in ways that are safer to people and the environment.

359 Development of a Non-Toxic Carbon Nanomaterial-Based Filter for Virus Sequestration in Aquaculture Systems

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Single-walled carbon nanotubes (SWCNTs) are highly sorptive materials that can potentially be used for environmental remediation of diverse water sources through removing pathogens. However, to our knowledge, the interactions between select pathogens, namely aquatic viruses and SWCNTs have not been well studied. We hypothesized that SWCNTs will sequester and inactivate a model fish virus that has relevance to the aquaculture industry which will mitigate infectivity to fish. The overall goal is to develop a filter that can be used by aquaculture facilities to minimize economic losses due to pathogens while also protecting ecosystem and human health. For our approach, SWCNT suspensions were prepared by sonication in DMSO and vacuumed deposited onto 10 µm pore Omnipore membranes (Millipore) at loadings of (0.35, 0.7, and 1.0 mg SWCNT/cm^2). After extensive washes with ethanol and water, suspensions of largemouth bass virus (LMBV) were passed through the filter followed by additional washes. Expression of the major capsid protein of LMBV was measured by qRT-PCR as a measure of viral retention. Results indicate that SWCNTs can more efficiently remove and retain LMBV compared to bare filters and activate charcoal by up to 50% and that this removal is dependent upon the mass of SWCNTs present on the filter. Additional studies investigated how a range of water chemistries (pH and hardness) relevant to aquaculture facilities in Florida influenced LMBV removal. We are currently testing the efficacy of this SWCNT-LMBV interaction to limit LMBV transmission through waterborne exposure while assessing SWCNT release and toxicity. While our group has previously shown SWCNTs are not acutely toxic to fish they may cause more subtle sub-lethal effects based on their ability to interact with various endogenous and exogenous molecules in the environment and in organisms. The current study has far reaching implications for the potential use of this technology for removal of pathogens and other aquatic contaminants in a safe way.

Fate and Effects of Metals: Marine Issues

360 Ocean acidification alleviates mercury toxicity to the life history traits in Tigriopus japonicus under multigenerational exposure scenario

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Ocean acidification (OA) and mercury (Hg) pollution are frequently concurrent problems in marine and coastal environments. And yet, very little information is known about the impact of OA on Hg toxicity to marine organisms, let alone under long-term multigenerational scenario. Here, we investigated the effects of OA and Hg pollution (i.e., ambient condition: pCO_2 400 ppm × control; and stress conditions: pCO_2 400 ppm × 10 µg/L Hg, pCO_2 1000 ppm × control, p CO_2 1000 ppm × 10 µg/L Hg) on development and reproduction of marine copepod Tigriopus japonicus under a multigenerational life-cycle exposure of four successive generations (F0-F3). Metal accumulation, as well as seven life history traits, that is, developmental time for nauplius phase, developmental time to maturation, survival rate, sex ratio (F/M) number of clutches, number of nauplii/clutch and fecundity was analyzed for each generation. The results indicated that, compared to single Hg exposure, CO_2 acidified seawaters led to a significantly lower Hg accumulation in adult copepods under the combine exposure, furthermore, with a trend for higher Hg accumulation from F0 to F3. Under most circumstances, OA exposure displayed negligible impacts on the seven traits in the copepod. Additionally, only number of nauplii/clutch was evidently suppressed by Hg treatment. Interestingly, OA could significantly alleviate the Hg inhibitory impacts on number of clutches, and number of nauplii/clutch, and it was attributable to less metal accumulation under the combined exposure than that under the single Hg treatment. Also, the combined exposure shortened the development time in contrast to the Hg exposure alone. Overall, OA could mitigate Hg toxicity to some important life traits in marine copepods during the long term exposure, and environmental risk assessment of Hg pollution must therefore integrate the elements in global climate change (e.g., OA) so as to provide a realistic measurement of the influences on aquatic ecosystem.

361 Antioxidant responses in relation to subcellular partitioning profiles of metals in transplanted clams: Implications for metabolic availability

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Due to the complexity of heterogeneous sediment, the chemical speciation and mobility of metals were in dynamic conditions rather than steady states, resulting in high variability of contaminant bioavailability. Chemical analyses in traditional tests had disadvantages of inaccuracy for contamination characteristics and risk assessments, representing a worst-case scenario in terms of complex exposure dynamics. In this presentation, relevant scientific issues regarding the effects on subcellular distribution and molecular biomarkers with special emphasis on toxic mechanisms and their associations were investigated through powerful experimental designs and field-based manipulations of in-situ exposure and kinetic DGT approach. Understanding the degree of pollution and geochemical characteristics of sediment matrix, in-situ testing chambers were deployed combining the DGT and caged clam Ruditapes philippinarum, and the dynamic exchanges, translocation and mobilization were then clarified among the interface of particles and pore-water in sediments through measuring dynamic parameters and resultant induced fluxes in exposed organism. Integrating a wide battery of biomarkers, dynamic changing processes were assessed, and their interactions were established between exposure and biological effects. Furthermore, variations of transcript expression of functional genes in contaminant-specific biomarkers were obtained through high throughput oligo-DNA Microarray and quantitative reverse transcription polymerase chain reaction (Q-RT-PCR) in order to elucidate the mechanistic understanding of molecular biomarkers in biological responses. Simultaneously, the approach of in-situ evaluation was established considering the consistency of framework as protocols among sediment chemistry, contaminant bioavailability and adverse effect, which significantly improve accuracy and ecological relevancy in complex exposure situation and thus provide a robust tool to support more comprehensive processes of sediment risk assessment. Overall, the associations not only revealed the fates of accumulated metals, but scientifically favored an improved understanding of toxic effects in response to subcellular level, supporting the focus of metabolic availability assessment on the intracellular processes or events occurring within organism in future coastal biomonitoring. (The author acknowledge financial support by Grant No. 21377125/B070403 from National Nature Science Foundation of China)

362 Detailed Reconstruction of the Metal Exposure History of Whales from Chemical Analysis of Earwax Plugs

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Baleen whales, including fin whales, are known to accumulate cerumen or earwax from birth to death forming an earplug. Earplugs often contain alternating light- and dark-colored laminae which can be used to provide age estimates for the individual with approximately six month resolution. Whale earplugs have also been used to reconstruct lifetime chemical profiles in whales. This reconstruction technique combines age estimates (counting light and dark lamina) with chemical analysis (i.e. measuring organic compounds) in each lamina. Published reconstructions have focused on organic contaminants, hormones, and total mercury (THg). Here we present an analytical method capable of measuring a very large suite of metals including THg and methylmercury (MeHg) in whale earwax laminae and document method performance in a 4-lamina earwax plug from a young fin whale. We applied an automated mixed-acid microwave-aided digestion protocol to completely solubilize sub-samples (100 mg) of the laminae, followed by magnetic-sector ICPMS analysis for quantification of 45 elements. Separate sub-samples of earwax were processed for THg and MeHg analysis with cold vapor atomic fluorescence (CVAF) detection. Mercury analytical recoveries were quantitative and method blanks represented < 1% of a typical earwax sample. The major inorganic constituents of the fin whale earwax laminae were Ca and P (averaging 29 mg/g and 15 mg/g respectively), followed by Na (3.8 mg/g), K (0.59 mg/g), and Mg (0.43 mg/g). Three transition metals, Zn (22 µg/g), Fe (16 µg/g) and Cu (12 µg/g) ranked next in concentration. Levels of most other transition metals/semi-metals (3 to 40 ng/g) and rare earth elements (0.03 to 4 ng/g) were significantly lower. THg concentrations increased consistently from lamina 1 (10.4 ng/g) to lamina 4 (18.2 ng/g) a nearly two fold change. MeHg concentrations tracked those of THg (r = 0.98), increasing from 5 ng/g to 11.6 ng/g, representing 48% of THg in lamina 1 and 62-67% of THg in laminae 2-4. Mercury levels were strongly anti-correlated (r = > -0.8, r = > -0.95 for Ca and P) with the major inorganic elements, suggesting dilution of the mercury source(s) by bulk components. Mercury levels were however strongly positively correlated with Al, Cd, Pd, Pt, and V, all elements (with the exception of Cd) associated with catalysts and fuel oil combustion. Carbon and nitrogen stable isotope analyses are being performed to help identify diet sources and changes.

363 Understanding the complex effects of salinity on metal toxicity using toxicokinetic-toxicodynamic model as a framework

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In estuarine waters, salinity is one of the most important factors that determine the toxicity of metals. The multiple effects of salinity include: changing metal speciation, providing competing cations and altering the physiology of organisms. Separation and quantification of these effects can provide better understanding of the complex effects of salinity. In this study, salinity effects on the toxicity of two metals (i.e., Cd and Cu) in the estuarine clam, Potamocorbula laevis, were delineated using the toxicokinetic-toxicodynamic model. Metal bioaccumulation at salinities ranging from 5 to 30 psu were quantified using a stable isotope tracer technique; in parallel, 96-h (for Cd) or 72-h (for Cu) toxicity tests were conducted also at those salinities. With the increase of salinity, the bioaccumulation of both Cd and Cu decreased monotonically, consistent with the stronger complexation effects of anions (e.g., Cl^- and HCO_3^-) and the competition effects of major cations (e.g., Na^+, Ca^{2+}). In contrast, increasing salinity led to different effects on the toxicity of the two metals, i.e., decreasing the toxicity of Cd, while increasing the toxicity of Cu. Toxicodynamic analyses showed that increasing salinity lowered the sensitivity of the clams to the internalized Cd (i.e., higher toxicity threshold and lower killing rate of Cd), whereas elevated the sensitivity of clams to the internalized Cu. This difference was presumably due to the different roles of the two metals in disrupting osmoregulation. The observed salinity effects in the toxicity tests were a combination of the effects on metal bioaccumulation and organisms' sensitivity to the internalized metals, and thus showed different patterns in different cases. In conclusion, the toxicokinetic-toxicodynamic model provided a useful framework for explaining the complex effects of salinity on metal toxicity, and can be used for better risk assessments of metals in estuarine waters.

364 Using the starlet anemone, Nematostella vectensis, for the assessment of effects and recovery in acute studies: development of a stress-response index

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Cnidarians have not been routinely used and recovery is not a typical endpoint in aquatic toxicity studies to assess the effects of metal exposures on native species. We have developed a unique acute toxicity test with the starlet anemone, Nematostella vectensis, to evaluate general stress, toxicity, and recovery post exposure to metals. Juvenile anemones were used in acute toxicity studies with cadmium and copper and were subsequently held in clean control water for a duration of two weeks post-exposure to determine if highly stressed anemones were able to recover from potentially lethal exposures. A stress-response index matrix of macroscopically observable conditions was developed including parameters such as: anemone column shape, color, and condition; mesentery visibility and condition; tentacle shape and level of retraction; tissue color and level of opaqueness; and response to physical or optical stimuli. Anemones were assigned to a numerical level of stress based on exposure-effect relationships and conditions were monitored during the two week recovery process. Any change in anemone condition was recorded, all anemones were re-ranked at the end of the recovery duration, and toxicity endpoints were recalculated. Impact is demonstrated by comparing concentration-response curves for exposures pre- and post- recovery. When using novel or indigenous species in toxicity studies with limited toxicity data available in the literature, we recommend similar studies to understand the potential for recovery of those organisms.

365 Cellular biomarker responses in Aurelia aurita exposed to various copper concentrations as a novel pelagic & estuarine bioindicator

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Jellyfish are ubiquitous in marine systems, ranging from shallow bays and estuaries to great depths of pelagic areas. Due in part to the complexity of scyphozoan life histories, even slight changes in environmental factors can have an effect on fecundity, development and population fluctuations. Anthropogenic activities have been suggested as a major cause of increases in jellyfish. These include overfishing, eutrophication, and habitat modification. In addition, jellyfish, like other aquatic organisms, are exposed to a host of chemical contaminants in the ambient environment. Heavy metals, such as copper, are a major anthropogenic pollutant found throughout aquatic ecosystems. However, there are few accounts of copper contaminant effects on scyphozoans in terms of biochemical markers. This study evaluated a host of sub-lethal cellular biomarker assays combined with behavioral analyses to determine the effects of 48 hr. exposures to 5, 10, 25 and 50 ppb Cu concentrations on Aurelia aurita, the common moon jellyfish. No adverse effects were seen in medusae at 5 or 10 ppb. Results showed the lysosomal destabilization assay was the most sensitive assay when compared to glutathione and lipid peroxidation. Results for lipid peroxidation and total glutathione varied from exposure to exposure in both oral arm and bell tissues. Lipid peroxidation did not vary significantly as Cu concentration increased. Glutathione levels did increase as Cu concentration increased, suggesting that increased antioxidant capacity (glutathione) inhibited increased cellular damage by peroxidation of lipids. Behavioral differences were also observed as Cu concentration increased--decreased pulsation rate and a decline of suspension in the water column. Biochemical markers provide useful tools in determining molecular responses to contaminants that may have effects on A. aurita populations and are valuable for identifying species-specific sensitivities. These tests, coupled with behavioral analyses, can then further be used to assess ecological implications at the community and population levels.

366 Zinc and calcium pathways in the green crab Carcinus maenas

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Green shore crabs (Carcinus maenas) have been extensively used to determine impacts of metal contaminants, because of the high capacity of their carapace to retain metals from the aqueous solutions. The relationship between metals and ions provides an interesting focus since the crabs demand high amounts of Ca^{2+} and also because metals may possibly be taken up by Ca^{2+} transport pathways. This demand is directly related to carapace formation and molting which may mobilize Ca^{2+} to/from the carapace. We studied the pathways by which Ca^{2+} and Zn are taken up from ambient sea water by the green shore crabs (Carcinus maenas), by investigating the relative contribution of uptake through the gill versus uptake across the carapace. Alive, dead and carapace-covered crabs were exposed to radiolabelled Ca^{2+} and Zn individually for 24 h at 2°C, 12°C and 22°C and the spatio-temporal distribution of each element was determined. Our results indicate that Zn uptake pathway occurs mainly through the gills (~75%) and via biosorption in the carapace surface (~25%), while the Ca^{2+} uptake pathway is via the gills (~90%). At 24 h, radiolabelled Zn was mostly concentrated in the gills (~70%), while Ca^{2+} was mostly concentrated in the carapace (~60%). No change was observed between 2°C and 12°C. An increase of Zn in the gills (~290%) and both ions in the carapace (Ca^{2+}= ~280%; Zn= ~300%) was observed at 22°C relative to 12°C. Despite the increased uptake of the two metals, the pathway of Zn and Ca^{2+} uptake remained similar to 12°C (via gills, ~73% and ~88% respectively). However, the pathway of Zn deposition in the carapace differed. The deposition occurred via biosorption (~65%) and through the gills (~35%) followed by redistribution to the carapace (IDRC, NSERC Discovery).

367 Development of the marine amphipod Parhyale hawaiensis as a model organism for ecotoxicology

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Brazil has a huge abundance of surface water resources and many of these are threatened by anthropogenic pollutants. Investigation of the effects of pollutants on aquatic life is critical; however, there is a lack of ecotoxicity test organisms that are native to Brazil. The amphipod Parhyale hawaiensis is an important marine organism in Brazil and offers considerable potential to become a model for ecotoxicology. This amphipod is already a model organism in developmental and genetic studies and numerous tools are available for its use in ecotoxicology. Although some toxicity tests have been conducted with Parhyale, information regarding standardized methods, responses to reference toxicants, and test protocols are limited. Within our research program on the development of Parhyale for ecotoxicology, this study investigated the use of a miniaturized (96-well plate format) protocol to assess the acute toxicity of zinc, copper, silver, and cadmium. In addition, procedures for measurement of changes in expression of biomarker genes [Glutatione S-transferase (GST) and metallothionein (MT)] by quantitative PCR, and biomarker protein level (metallothionein) quantification by reversed-phase high-performance liquid chromatography were developed. Toxicity tests demonstrated the feasibility of the proposed protocol and showed that P. hawaiensis presents metal sensitivity in the range of other marine amphipods. LC50 (96 h) values were 1.63, 0.55, 3.73 and 1.09 mg L^{-1} for Ag, Cd, Cu and Zn, respectively (or 15.1, 4.93, 58.7 and 16.7 µM for Ag, Cd, Cu and Zn, respectively). Gene expression analyses and metallothionein quantification are still underway. This is the first report regarding metals toxicity and induction of toxicological biomarkers in this organism.

Science of Sediment Toxicity Testing: Method Advances, Interpreting Results and Use of Data in Ecological Risk Assessments

368 Analytical Challenges for Quantification of Test Materials in Prospective Sediment Toxicity Laboratory Investigations

D.A. Koch, ABC Laboratories, Inc.; J. Rhodes, ABC Laboratories

A key component of laboratory sediment toxicity testing for benthic organisms is the accurate measurement of the distribution of the test material between the sediment, pore water, and overlying water. For compounds having some degree of toxic effect, the lower concentrations which must be measured to elucidate dose response data present inherent challenges to the analytical chemist. Because of the unique nature of the sediment toxicity testing analytical measurement endpoints, the availability of applicable analytical methodology, in advance of this testing requirement being triggered, is not always a given. At times, the development of methods that can measure test compound concentrations at extremely low levels will be approaching the limits of currently available instrumental sensitivity. In particular, highly hydrophobic compounds can exacerbate the difficulty of the water measurements, as most of the compound will partition into the sediment phase, leaving very little to be measured in the water fractions. Practical approaches to these challenges will be discussed, along with possible strategies to employ when the measurements are particularly difficult for the levels being testing. Some representative strategies for the analysis of hydrophobic compounds in aqueous matrices will be presented.

369 SEDAG Spiked Sediment Database: A New Resource for Sediment Toxicology Data Discovery and Synthesis

S.M. Bay, Southern California Coastal Water Research Project / Toxicology Dept; P.L. Myre, Exa Data & Mapping Services; A.S. Bess, Chevron Energy Technology Company / Environmental Unit

Sediment toxicity is a key line of evidence for sediment quality evaluation and ecological risk assessment. Synthesis and analysis of field sediment toxicity data in the 1990's and 2000's resulted in the development of sediment quality guidelines (e.g., ERM, PEL) that are widely used to interpret sediment chemistry data. Most of these past synthesis efforts have focused on environmental sample toxicity results. Few resources are publicly available for discovery and use of spiked sediment toxicity test data, which are essential for stressor identification, developing models to predict contaminant bioavailability, and improving sediment quality guidelines. The SETAC Sediment Advisory Group (SEDAG) has coordinated a collaborative effort over the past two years to develop a web-accessible database containing results from spiked sediment toxicity tests. This effort has been successful, resulting in the development of a database structure, completion of the initial phase of database development, and creation of a web-accessible interface for accessing the data. This initial version of the database contains results from over 200 studies that have been compiled into a standardized format including information on test species, duration, toxicity response, spiking method, and sediment characteristics (e.g., TOC, grain size). Multiple effect endpoints (e.g., LC50, EC25, LOEC, NOEC) for over 100 types of chemicals, including metals, chlorinated pesticides, pyrethroid pesticides, and petroleum compounds are included. The database interface allows public access via internet browser and contains features to allow the user to design a customized query and download the data. Future plans for database development and maintenance will be described, including collaborative efforts for curation and addition of additional records.

370 The Challenges Associated With Achieving Freshwater Sediment Toxicity Testing Standardization in Australasia

W.T. Mehler, V.J. Pettigrove, Univ of Melbourne / Zoology

As stated in the description for this SETAC session – "Various acute, short-term, and chronic toxicity testing guidelines for freshwater (Chironomus, Hyalella) and marine (Leptocheirus) organisms are available." This statement, as based on North American species, also rings true, for much of Europe as well, as OECD and ASTM guidelines are available for both European native midges and amphipods (e.g. Chironomus riparius and Gammarus pulex). Unlike these developed countries, sediment toxicity testing (especially for freshwaters – which is the focus of this presentation) is still in its infancy in much of Australasia. The use of the aforementioned sediment toxicity testing guidelines is somewhat of limited use as these freshwater test organisms are not indigenous to these areas. This presentation will provide a snapshot of the current status of freshwater sediment toxicity testing in Australasia as well as the current challenges that standardization of freshwater sediment toxicology faces this area of the world.

371 Characterization of sediment chemistry, sediment toxicity and macroinvertebrate communities in wadeable streams of the Southeastern United States

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As part of the US Geological Survey's (USGS) National Water-Quality Assessment (NAWQA) project, sediment samples from streams representative of an urban gradient and selected agricultural lands were evaluated from the southeastern United States (AL, GA, NC, SC, VA) in the summer of 2014 (DOI: 10.3133/ofr20151095). The study evaluated relationships between sediment toxicity, sediment chemistry (metals, pesticides, organochlorines, and polycyclic aromatic hydrocarbons) and co-located macroinvertebrate communities at 59 of those sites. Sediment toxicity was evaluated by conducting whole-sediment laboratory toxicity testing with the amphipod Hyalella azteca (28-d exposure), the midge Chironomus dilutus (10-d exposure), and the mussel Lampsilis siliquoidea (fatmucket; 28-d exposure) in accordance with ASTM and USEPA methods. Toxicity endpoints included survival, weight, and biomass. Mean control survival and growth at the end of the exposures met test acceptability criteria. Roughly half of the sediments were identified as toxic to at least one of the test species, based on a significant reduction of at least one endpoint relative to the control sediments. Amphipod survival, biomass, and weight declined significantly as the percentage of urban land within the basin increased. Concentrations of metals, PAHs, a few organochlorines and current use pesticides infrequently exceeded individual sediment quality guidelines (i.e., Probable Effect Concentrations (PEC) or recent Likely Effect Benchmarks (LEB) for pesticides). A Reference Envelope approach was also calculated that tended to increase the sensitivity of the toxicity endpoints due to strong and consistent control and reference site performances. Evaluation of concentrations relative to their benchmarks, both individually and as summed PEC or LEB quotients, was explored on both a carbon-normalized and a dry-weight basis. The use of these concentrations and benchmark exceedances in explaining both toxicity and macroinvertebrate communities co-located at these sites are presented.

372 Resolving Sediment Toxicity for Ecological Risk Assessment at Complex Urban Waterways

D.P. Hennessy, J. Volosin, Anchor QEA LLC; L. Logan, Anchor QEA; D.H. Haury, Anchor QEA LLC; D. Glaser, Anchor QEA

As part of the Baseline Ecological Risk Assessment for the Newtown Creek Remedial Investigation, 28-day sediment toxicity tests were completed for 36 creek stations and 24 reference area stations, using the amphipod Leptocheirus plumulosus. Reference area data were used to develop a reference envelope for comparison with the site that reflected the range of industrial and municipal point source/combined sewer overflow (CSO) impacts at the site. Bulk sediment was analyzed for more than 200 analytes and conventional parameters, as well as acid volatile sulfide (AVS) and simultaneously extracted metals (SEM), and pre- and post-toxicity testing, to evaluate metal bioavailability. Contaminant bioavailability was also evaluated by synoptically collecting porewater for polycyclic aromatic hydrocarbon (34) (PAH [34]), polychlorinated biphenyl congeners, and pesticides using solid-phase microextraction fibers, and for metals using mini-peepers. Concentration-response models were developed that included reference envelopes representing the 95% lower confidence limit on the 5th percentile for survival, growth (biomass and weight), and reproduction (per surviving amphipod and per surviving female amphipod). Using these models, a majority of the toxicity could be explained by porewater contaminant concentrations. For a number of locations, toxicity is likely due to PAH (34), with some contribution from metals. AVS and SEM indicated low bioavailability of cadmium, copper, lead, nickel, and zinc, with no change in bioavailability pre- and post-toxicity testing. However, for some stations, the cause of toxicity could not be resolved based on porewater contaminant concentrations or standard confounding factors such as sulfide, ammonia, and grain size. Given the proximity of these stations to municipal stormwater outfalls and CSOs, it is surmised that the unresolved toxicity may be expressed by other components of urban runoff or municipal discharges, which are currently under investigation. These findings strongly support the need for further evaluation of the factors confounding sediment toxicity test results in complex urban waterways.

373 Predicting sediment metal toxicity to Hyalella azteca using a metals mixtures version of the Biotic Ligand Model

R.C. Santore, K.E. Croteau, A.C. Ryan, Windward Environmental LLC

The Biotic Ligand Model (BLM) is a computational framework used for predicting the effects of water chemistry on metal bioavailability. In this presentation we describe the development of an extension of the BLM framework that can be applied to mixtures of multiple metals (the mBLM considers Cd, Cu, Ni, Pb, and Zn). The mBLM was used to predict toxicity in chronic exposures with Hyalella azteca. Calibration of the mBLM to H. azteca was accomplished by comparison with water-only toxicity tests. Equilibrium partitioning theory predicts that the toxic effect that may result from exposure to sediment pore-water is similar to effects resulting from exposure to the water column. The calibrated model was then used to predict whether field sediments from several sites characterized by elevated metal concentrations would be toxic to H. azteca. Predicted toxicity was compared with measured toxicity in chronic tests with H. azteca from several field sites and high predictability was observed. The mBLM analysis allows sediments to be ranked to identify which samples would be most likely to cause toxicity. The mBLM can also identify which metals in the mixture of metals are most likely to be responsible for any observed toxicity. The overall good performance of the mBLM suggests that it is a powerful predictive tool for use in assessing risk from metal concentrations in field sediments and could be used as part of a general methodology in the development of sediment guidelines.

374 Evaluating the relative sensitivity of endpoints generated during midge life-cycle for US FIFRA sediment toxicity test requirements-Part II

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Currently, lethality-based endpoints for chironomid (midge) life-cycle experiments described in USEPA test guidelines include larval, pupal, and adult survival, while sublethal endpoints include larval growth, adult emergence (total/percent, cumulative rate, time to first emergence, time to death, and sex ratio), and reproduction (time to oviposition, mean eggs/female, egg cases/treatment, and egg hatchability). High variability in control response and redundancy of information gained from similar observations prompted questions about the utility and/or necessity of certain endpoints for defining biological effect thresholds. This research is a continuation of that presented by the CropLife America Ecotox Work Group Sediment team at the SETAC North America 2015 meeting that detailed insight gained following the compilation of recen midge toxicity studies into a database. Meta-analysis of the data demonstrated that larval endpoints (survival and growth) and adult emergence consistently established the lowest NOEC values for individual studies, whereas reproductive endpoints and adult survival were always less sensitive. This presentation continues to focus on the relative sensitivity of endpoints, but more closely examines inter- and intra-replicate variability as well as the presence or absence of dose-dependent responses. Based on further analysis of information in the CropLife America database, coupled with considerations related to the effort required to gather endpoint data, suggestions for streamlining the number of measured endpoints to improve test method efficiency and provide researchers with greater confidence that observed effects are indeed related to contaminant exposure will be discussed.

375 EPA's Method for the Amphipod, Hyalella azteca, Water-only Exposures for Acute, Short-Term Tests, and Chronic Toxicity Tests

T.J. Norberg-King, T.L. Highland, R. Hockett, D.R. Mount, USEPA / ORD NHEERL Mid-Continent Ecology Division

Hyalella azteca, a freshwater amphipod, is a common organism used for sediment toxicity testing in North America. USEPA/ASTM and Environment Canada have standard methods for short-term and longer-term sediment toxicity tests with H. azteca. However, a protocol has not been published or updated recently by USEPA/ASTM that provides guidance for the water-only testing of this species. Programs for registering chemicals, monitoring effluent discharges and receiving waters, and developing water quality guidelines have a need for data from additional freshwater invertebrates, particularly those that can be used to derive chronic effect data. At EPA-Duluth, we have successfully performed chronic water-only tests with H. azteca through reproduction, and these long-term tests can be adapted for shorter durations, such as the 28-day exposures with H. azteca (measuring growth and survival). We will present the methods that we currently use for tests of various durations, including 96-h, 10-d, 28-d, and full chronic tests of 42- to 49-d. Disclaimer: This presentation does not necessarily reflect the views or the policies of the U.S. Environmental Protection Agency.

Assessing Contaminant Effects in Multi-Stress Ecosystems – Part 1

376 Can a comprehensive understanding of real drivers of ecological degradation be drawn from presently available data? A case study for Europe

I. Rodea-Palomares, Univ of Florida / IFAS / Agricultural & Biological Engineering; R. Muñoz-Carpena, Univ of Florida / Agricultural Biological Engineering

During the last decades, Ecotoxicology has drawn the basic cartography of chemical pollution and its potential ecological impacts. However, these maps are still fragmented and not ready to answer apparently simple questions from the real world such as: what is the main driver on the observed ecological alterations at a certain scale?; is it organic chemical pollution or other factor such as just nutrient enrichment or hydrological alteration?; in a common scale metric, which of these factors are more important? Few studies addressed large-scale ecological effects of chemical pollution, and even fewer are examine the relative contribution of organic chemical pollution in the context of other relevant chemical (such as eutrophization, acidification, salination, etc.) or non-chemical (such as hydrological alterations, erosion, irrigation, land cover changes, etc.) factors. Data scarcity, data fragmentation and the unavailability of chemical pollution remote sensing data hampers such large scale integrated studies. The present talk will cover the aims, methods and preliminary results of a project that aims to infer large-scale patterns of ecological degradation that can be asigned to chemical pollutants in the context of other potential stressors. This will be addressed with a novel integration of data mining, mechanistic modelling and GSA/UA analysis. The ultimate aim is to build relative importance map for different chemical pollutats in the context of other ecological stressors at the continental scale (Europe). The project mines data from the largest database of chemical pollution in Europe made available by the European Commision (more that 4000 monitoring stations, with more than 140 monitored chemical pollutants). In addition we used publicly available remote sensing data (climatic information, land cover, vegetation, etc.), global biodiversity data (Global Biodiversity International Facility) and other sociological, hydrological and geomorphological data sources to inform the analysis. We will critically analyse the characteristics of available data, their strenghts and weaknesess, the inferential approaches followed and the results obtained. Results from this analysis will be of interest to valuate the suitability of present data sources to inform large-scale integrated analysis and to identify structural gaps and defects in present monitoring strategies that may hinder or limit the potential success of such large scale analysis.

377 Urban runoff differentially affects coho and chum salmon spawners

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Urban stormwater runoff contains a complex mixture of contaminants that enter receiving waters when it rains. This mixture can cause a variety of toxic responses in fish and invertebrates, including mortality and cardiovascular abnormalities. In the Pacific Northwest, stormwater runoff causes pre-spawning mortality (PSM) in adult coho salmon (Oncorhynchus kisutch) returning to spawn in urban-impacted streams. Within a few hours of exposure, symptoms progress from lethargy and disorientation to loss of equilibrium, immobility, and eventually death. Although we do not know the precise cause of PSM, we previously determined that coho PSM is linked to storm events. Chum salmon (O. keta) spawning runs often overlap with coho. To determine whether chum are similarly susceptible to PSM, we co-exposed pre-spawn adult coho and chum to urban road runoff or well water in controlled exposures for 6 storm events. We monitored water quality and individual behavior throughout exposure. Exposures were terminated after 4 h or when fish became symptomatic. Only coho exposed to runoff became symptomatic. Across the 4-h exposure, chum appeared behaviorally unaffected by urban runoff. Furthermore, a point-of-care blood analyzer was used to measure ion concentrations and gases, pH, hematocrit, glucose, and lactate in arterial blood. Multiple blood chemistry parameters were affected in runoff-exposed coho compared to control coho, leading to the hypothesis of metabolic acidosis as the cause of death. In contrast, chum exposed to runoff were much less sensitive than controls.

378 Climate change and contaminants: A recipe for trouble

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Global climate change (GCC) is forecasted to increase water temperature and salinity in many aquatic ecosystems with predicted impacts to biodiversity within these systems. Synergistic effects between climate and other anthropogenic threats, such as water pollution, will likely intensify climate-change impacts. As climate change accelerates, pesticide use is predicted to increase to combat insect-pests and diseases, potentially affecting non-target organisms in sensitive habitats. Our previous research has shown that exposure to contaminants may elicit toxic effects even at concentrations below the limit of detection. As such it is critical to understand how organisms will respond to GCC stressors in the presence of contaminants. We exposed the amphipod Hyalella azteca to a matrix of temperatures and salinities ranging from 12 to 18°C and 0.2 to 8 parts per thousand (ppt), respectively, in combination with a low-level environmentally relevant concentration of the pyrethroid insecticide bifenthrin (1 ng/L) for 24h, 48h, and 96h. Cellular stress gene responses were monitored at all three time points using quantitative Polymerase Chain Reaction (qPCR). After 96h of exposure, an additional set of replicates was used to investigate changes in swimming behavior using the Ethovision video imaging software DanioScope and effects on growth among treatments and compared to controls. Our results demonstrate that compared to control conditions, the GCC related stressors salinity and temperature, result in negative effects to invertebrate motility, growth, and cellular stress gene response. However, the effects on all three endpoints are significantly more pronounced in the presence of bifenthrin compared to salinity and temperature alone. In addition, survival was significantly decreased in pyrethroid treatments combined with salinities of 4 and 8 ppt compared to treatments without the pyrethroid. These environmentally relevant exposures using the standard test species H. azteca provide essential information for understanding effects caused by GCC in conjunction with increasing pesticide use, further highlighting the need to incorporate GCC impacts into risk assessments of contaminants of concern.

379 Potential Effects of Pesticide Mixtures on Stream Quality in the Midwestern United States

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Pesticide occurrence and ecological condition were characterized in 100 wadeable streams in the Midwestern U.S. during May-August 2013 by the U.S. Geological Survey's National Water-Quality Assessment (NAWQA)

Project, in collaboration with the U.S. Environmental Protection Agency. Twelve weekly water samples and extracts from passive Polar Organic Compound Integrative Samplers (POCIS, deployed for about 5 weeks) were analyzed for 227 pesticide compounds (including degradates) at each site. Pesticides also were analyzed in daily composite water samples collected throughout the study period by microautosamplers at 7 streams. At the end of the study period, depositional bed sediment was sampled and analyzed for 118 pesticides and sediment-source tracking radioisotopes, and habitat and benthic invertebrate communities were surveyed. Complex pesticide mixtures were observed in weekly water samples (median 25 compounds) and in POCIS extracts (median 62 compounds). Herbicides and many degradates were detected in >90% of samples. Insecticides (often a single compound, especially imidacloprid) dominated the Pesticide Toxicity Index (PTI) in water for invertebrates, which reflects both concentrations and acute toxicity of pesticides in a mixture. Pesticide results for daily composite water samples showed that weekly discrete water samples frequently missed pesticide-concentration peaks, including insecticides present at potentially toxic levels. Compared to agricultural streams, urban streams tended to have higher PTI values, detection frequencies of most insecticides and fungicides in water, insecticide concentrations (bifenthrin and DDE) in bed sediment, and percentage of surface-derived bed sediment. The potential importance of pyrethroid insecticides was indicated by the predominance of bifenthrin in the sediment-PTI and by invertebrate community multistressor models, in which pyrethroid degradates in POCIS (cis-cyhalothric acid, 3-phenoxybenzoic acid) were significant explanatory variables. Mesocosm experiments supported these findings by showing that exposure to bifenthrin in suspended sediment caused effects on invertebrate communities that were consistent with bifenthrin concentrations and invertebrate community condition observed in Midwestern stream sediments.

380 Proteomics and Transcriptomics analyses of Florida Manatee (Trichechus Manatus Latirostris) Mortalities in 2013

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In 2013, two large-scale Florida manatee (Trichechus manatus latirostris) mortality episodes were associated with 830 deaths and a substantial population loss. The mortality episodes occurred on separate coasts of Florida, near Fort Myers on the west coast and in the Indian River Lagoon (IRL) on the east coast. The IRL mortality episode was associated with an unknown etiology and the Southwest Florida episode was attributed to a persistent bloom of red tide (Karenia brevis) algae in the region. Proteomics experiments on serum from healthy and impacted manatees indicated effects to the immune system, including proteins involved in inflammation and wounds and injuries. Several protein biomarkers were elevated in manatees from the unknown mortality compared to health manatees including complement C4-a isoform 1 (average ratio 1.25), histidine-rich glycoprotein (1.34), kininogen-1 isoform 1 (1.38), properdin (1.30), and protein AMBP (1.38). In the group of manatees recovering from red tide, several proteins were elevated including, complement C3 (1.42), complement C4-A isoform 1 (1.83), ceruloplasmin-like (2.32), angiotensinogen (2.08), and pyruvate kinase isozymes M1/M2 isoform 3 (2.29). These proteins are associated with acute-phase response, amyloid formation and accumulation, copper and iron homeostasis, the complement cascade pathway, and other important cellular functions and may serve as informative biomarker proteins for manatees. RNAseq was also performed on total RNA isolated from white blood cells comparing the red tide group to other manatees. The genes with the highest fold changes (FCs) and p-value < 0.05 were the OSCAR gene (3.68, p-value 0.0485), transmembrane protein 56-like (3.53 fold, 0.000036), myotubularin related protein 2, transcript variant 2 (MTMR2) (3.53, 0.0015), thymocyte selection associated family member 2 (THEMIS2) (3.38, 0.0005), matrix metallopeptidase 9 (MMP9) (3.32, 0.0450) and haptoglobin (HP) (3.28, 0.0013). Several genes were down regulated including many involved in the immune system. This work indicated that the immune system, leukocyte production and differentiation, neuronal activity and synapse and tumor invasiveness were major pathways targeted by red tide exposure.

381 Environmental Temperature Moderates the Effects of Estrogenic Exposure on Larval Fathead Minnows

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Exposure to wastewater estrogens been shown to adversely affect the survival and behavior of fish. However, evaluating the effects of estrogen exposure on individuals is complicated by the fact that rates of chemical uptake and environmental degradation are dependent on abiotic factors, such as temperature. A factorial experiment was conducted to assess the influences of temperature and estrogen concentration on larval fathead minnows (P. promelas). Larvae were exposed to 3 concentrations of estrone (low, high) or to an ethanol control, at 4 water temperatures (15, 18, 21, or 24 °C) for 21 days. Exposure-induced genetic and behavioral changes were assessed using established methodology for the quantification of fast-start (C-start) locomotive mechanics, a novel foraging efficiency behavioral assay, and gene expression analysis using SYBR-green RT-qPCR. A significant interaction between estrone concentration and temperature influenced predator evasion total escape response ($P = 0.027$), compared to control subjects, exposed subjects exhibited reduced escape performances at colder temperatures. Results for larval feeding efficiency did not show a significant interaction between temperature and estrone exposure, all larvae consumed prey at similar rates regardless of treatment. The analyses indicate that temperature has significant context-dependent modulating effects on behavioral interactions of exposed biota. Predicted scenarios for climate change, mounting pollution, and habitat loss continue to increase the environmental pressures on wildlife, therefore it is important to understand how abiotic factors moderate toxicant exposure during sensitive life stages of aquatic organisms.

382 Evaluating Stormwater Impacts on Coastal Sediment Recontamination

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Cleanup at contaminated sediment sites has often been initiated before land-based sources have been fully identified and controlled. Under such conditions, remediated sites may be re-contaminated by ephemeral inputs, e.g. from stormwater runoff which may contain high levels of solids-associated contaminants of concern (COCs). Historically stormwater assessment has been focused on loads rather than impacts on receiving sediments. Our objective is to develop and test techniques to assess the magnitude and characteristics of solid associated contaminants into receiving waters and sediments due to episodic storm events. Paleta Creek near Naval Base San Diego was selected for monitoring and modeling of stormwater and receiving environment response. Composite runoff samples were collected using auto-samplers, which were triggered at each location by site-specific criteria. The samples were analyzed for a variety of metals and organic contaminants as a function of particle size. Receiving waters were monitored for flow trajectory, sediment deposition and COCs. Sediments collected in outfalls, deposition traps and in the receiving environment were also subjected to chemical analysis and biological testing. The results show that the initial flush contained the highest COCs with zinc, fluoranthene and pyrene being among the dominant COCs. Over time the contaminant loadings decreased due to reduction in particulate contaminants while the concentrations in finer, colloidal and freely available size fractions remained relatively constant. Toxicity testing of stormwater runoff samples showed typical chronic toxicity, however, samples taken in the receiving environment over the course of the storm (~12hrs) showed decreasing toxicity with time. Toxicity testing of receiving sediments with amphipods also showed significant toxicity associated with stormwater sediment loads with increasing impairment as the wet season progressed.

383 Lessons Learned from the South River and Upper Shenandoah Series of Multiple Stressor Ecological and Human Well-being Risk Assessments

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We have conducted a series of regional scale risk assessments using the Bayesian Network Relative Risk Model (BN-RRM) to calculate the ecological and human well being risks to the South River and upper Shenandoah River study area abbreviated SSRA. The ecological endpoints were Smallmouth bass, White sucker, Belted Kingfisher and Carolina Wren and four abiotic endpoints; Fishing River Use, Swimming River Use, Boating River Use and Water Quality Standards. A further ecological risk assessment was performed incorporating two management options, bank stabilization and agricultural best management practices. The third risk assessment integrated human health and ecosystem services with the endpoints Human health, Water quality, Recreation, and the Recreational fishery. The final risk assessment in the series estimated the effects of an increase in temperature to three endpoints, smallmouth bass, white sucker and Caroline wren due to climate change in the 2071-2100 timeframe. Although mercury (Hg) contamination was the original imputes for the site being studied and remediation initiated, other chemical and physical stressors were critical and often were the largest contributor to risk depending on endpoint and location in the watershed. Bank stabilization does reduce risk to some of the endpoints but the design also needs to consider an increase in risk to belted kingfisher along bank habitats. The increase in temperature due to climate change did not increase risk to the three endpoints but in some instances reduced risk. In the integrated human health and ecosystem services assessment Hg was the first ranked stressor for human health. River temperature and E. coli were the major contributors to risk for water quality and recreational river use. The primary conclusion is that it is possible to build a risk assessment process using the BN-RRM approach that incorporates multiple types of stressor and endpoints over large spatial scales. Human health risk can be compared to those including water quality, biological endpoints and ecosystem services. The risk assessment process can also be incorporated into an adaptive management framework that incorporates governance, stakeholder engagement, decision making and the occurrence of Black Swans, factors such as climate change or unforeseen social or technological factors.

The Other Oil Spills

384 Effects of a petroleum spill on stream communities in West Creek, Colorado

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Oil development has expanded dramatically in Colorado over the last decade. As a result, there has been a significant increase in the number of accidental releases into the environment. In 2014, over 300 petroleum spills were reported to the Colorado Department of Health. The Unaweep Canyon spill in January 2013 discharged 22,700 liters of gasoline and 7,300 liters of diesel fuel into West Creek, a tributary to the Dolores River, acutely killing an estimated 1,200 brown trout and 8,200 mottled sculpin. Five subsequent electrofishing surveys over the following three years indicated that the fishery was not recovering, particularly with regard to mottled sculpin abundance. In June and October 2015 we explored stream health indicators across multiple levels of biological organization in order to determine the long-term effects of this spill. Significantly elevated white blood cell counts were detected in mottled sculpin collected from the spill site. Histopathological abnormalities including hepatocyte necrosis and hepatocyte cytomegaly and karyomegaly were discovered. Increased melanomacrophage aggregates were observed in older fish relative to young fish, with a greater disparity between the age classes at downstream sites. Occasional congenital anomalies were also identified (cystic renal tubular ectasia; neural choristoma) in mottled sculpin at the spill site and downstream. Altered benthic macroinvertebrate community structure was detected at the spill site compared with a reference site one kilometer upstream. Interestingly, GC-MS finger-printing analysis of polycyclic aromatic hydrocarbons (PAH) in stream sediment revealed that by 2015 PAH concentrations, at all sites, were typical of a minimally impacted stream flowing adjacent to a road. These results indicate that the biological effects of the spill were persisting after sediment PAH concentrations had returned to ‘normal’.

385 Lac-Mégantic Oil Spill and Disaster – Quality and Toxicity Assessment: Results, Difficulties and Concerns

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On the night of July 5, 2013, a freight train carrying light crude oil from North Dakota derailed in the town of Lac-Mégantic (Quebec, Canada), causing explosions, fires and the spill of close to 7000m^3 of oil. This catastrophe resulted in 47 human deaths, the destruction of the city’s downtown and significant environmental pollution. Indeed, oil infiltrated soils and contaminated Lac-Mégantic (lake) and the Chaudière River. Floating oil was observed up to 186 km from Lac-Mégantic in the direction of the St. Lawrence River. This emergency is considered the worst case of an inland oil spill in North America. Remediation actions included excavation and biotreatment of contaminated urban soils and monitoring of contaminated water and sediments along the Chaudiere River. Following the accident, quality assessments on water, soils and sediments within a 190Km corridor between the lake and river were undertaken by the Québec Government for the first two years. A multidisciplinary group of researchers from five of Quebec universities initiated a vast research program in order to use this case as a learning instrument for various environmental studies that included emergency actions and environmental impact evaluation. Within this second aspect, the group integrated and analyzed the data from the universities and government to give a more accurate portrait of the environmental short term and long term impacts following the disaster including chemistry, toxicity assessment and ecology. Special issues needed to be considered for these assessments given the presence of poorly known unconventional light crude from North Dakota that included a varied mixture of chemical additives. This paper aims to present the results concerning Lac-Mégantic’s soil’s and Chaudière River sediment quality and toxicity studies.

386 Diluted Bitumen (Oil Sands) Spills into Rivers--Lessons from the 2010 Enbridge Line 6B Pipeline Release into the Kalamazoo River

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New application of science is changing response strategies for spills of crude oil into freshwater ecosystems, especially for diluted bitumen spills. The Enbridge Line 6B pipeline release of diluted bitumen into the Kalamazoo River downstream of Marshall, MI in July 2010 was one of the largest freshwater oil spills in North American history. The unprecedented scale of impact and quantity of oil released required the development and implementation of new approaches for submerged oil detection and recovery. At the onset of cleanup, conventional recovery techniques were successfully employed for the initially floating oil. However, volatilization of lighter diluent, and mixing of oil with sediment during flooded, turbulent river conditions caused the oil to begin sinking within 10 days of the discharge. For the next four years, the cleanup would focus on the submerged oil that had collected in natural deposition areas along 38 miles of river corridor, including three impoundments. Total cleanup costs would eventually exceed a billion dollars. Recovery complexities for submerged oil mixed with sediment in depositional areas and chronic, spontaneous oil sheens along the river led to the development of a multiple-lines-of-evidence approach comprising six major components: geomorphic mapping, field assessments of submerged oil (poling), systematic mapping of oil sheen, hydrodynamic and sediment transport modeling, forensic oil chemistry, and net environmental benefit analysis (NEBA). The approach was developed through close communication between scientists and operations staff, especially concerning matters of prioritization and containment. Information from the integration of these six techniques was used by the Federal On-Scene Coordinator (FOSC) in determining the appropriate course of action for each impacted segment of the river. The NEBA allowed the FOSC to weigh the ecological risks associated with leaving residual submerged oil in place against potential negative effects associated with removing the oil. Eventually, dredging was determined to be the appropriate approach, mainly in the large impoundments where most of the submerged oil settled. The need for quick use of conventional recovery tactics, submerged oil containment, multidisciplinary science, and implementation of contingency planning for effective response organization are major lessons learned. All rely upon an understanding of the time continuum of diluted bitumen weathering processes.

387 The Montara Oil Spill, Timor Sea – Two Years of Fish Health Monitoring

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The Montara incident which occurred in 2009 in the Timor Sea, resulted in the release of an estimated 23,000 barrels of oil and gas condensate over 74 days. Following the operational response, the scientific monitoring focussed on commercially important fish in order to ascertain the suitability for human consumption, as well as inform on the long term effects of the oil spill on fish health. Red emperor (Lutjanus sebae, n = 807) and glodband snapper (Pristipomoides multidens, n = 1531) were sampled over 2 years flowing the accidental release, and biopsies collected. A suite of physiological indices (condition factor, liver somatic index and the gonadosomatic index), biomarkers (EROD activity, biliary polycyclic aromatic hydrocarbon (PAH) metabolites, liver integrity measured by serum sorbitol dehydrogenase activity (SDH), oxidative DNA damage) were measured. While fish initially showed signs of exposure to petroleum hydrocarbons, biomarker of exposure in fish collected in the most impacted area have returned to reference levels within 24 months. Only liver somatic index in fish collected at the spill site remained elevated two years after the spill, relative to fish from other locations. Two years of monitoring following the oil spill in a tropical open sea provided information on biomarker tools that are suited to short-term, or long-term monitoring of effects from discharged light crude oil.

388 Toxicity of organic fractions of hydraulic fracturing flowback and produced water (FPW) to early life stage of zebrafish

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The potential environmental risk related to horizontal drilling with high-volume hydraulic fracturing (HF) has drawn a lot of public concerns. However, the toxic effects on fish embryo development posed by HF flowback and produced water (FPW) has not been well studied. FPW is a complex mixture of organic and inorganic constituents in HF fluids and deep formation water. The surface spills of FPW may cause acute and/or long term environmental impacts on aquatic ecosystems. Previous studies indicated FPW may have endocrine disruptive and embryotoxic effects on fish. In this study, the total organic fractions were extracted from sediment-free water and sediment phases of several representative FPW samples. Analyses of PAH profile were performed for each organic fractions, demonstrating that FPW samples have complicated and highly variable organic profiles based on but not limited to PAHs profile results. Zebrafish embryos were exposed to various concentrations of organic fractions for 96 h. Compared to the previous studies using the dilutions of original FPW samples, the organic fractions of FPWs have much higher LC50 values, demonstrating that the acute toxicity is dominated primarily by high salinity in original FPW samples. However, exposure to organic fractions of FPWs significantly increased the incidences of morphological deformities in zebrafish embryo development, including pericardial edema, yolk sac edema, and spinal curvature. The Ethoxyresorufin-O-deethylase (EROD) assay using hatched larvae demonstrated significant increases in EROD activity in each exposure groups. Expression of a series of genes related to xenobiotic metabolism, oxidative stress and endocrine disruption were also found to be altered in exposure groups through quantitative real-time polymerase chain reaction. These findings provided novel information on the toxicity of organic contents in FPWs, and suggested that the organic contaminants released from FPW spills may have potential long-term environmental effects of FPW spills on teleost early development.

389 Developmental and endocrine responses in a freshwater fish (Oreochromis mossambicus) and amphibian (Xenopus laevis), exposed to old bunkered crude oil

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Crude oil pollution has been linked to health disorders in various aquatic vertebrate species. Most published research reported on marine organisms whereas little is known about the health effects of crude oil on freshwater aquatic vertebrates. In this study we used African clawed frog (Xenopus laevis), and Mozambique tilapia fish (Oreochromis mossambicus), as model organisms to study effects of crude oil on the endocrine systems of freshwater vertebrates. For exposures, we prepared water accommodated fractions (WAF) of crude oil and collected surface water samples downstream of an old coal mine bunker. Following short- and long-term exposures we used several in vitro and in vivo bioassays, including recombinant yeast assays, frog testis cultures, frog embryo-toxicity and teratogenesis assays, juvenile fish and tadpole exposure, to assess morphological, histological and molecular endpoints associated with several pathways, including thy thyroid endocrine system. The bunker crude oil WAF did not exhibit teratogenic potential nor embryo-toxicity. However, a higher malformation incidence was observed in frog larvae exposed to surface water collected downstream from receiving waters. The crude oil did exhibit both anti-androgenic and anti-estrogenic activity. Anti-androgenic activity was also recorded in surface water samples, as well as water samples collected from two oil/water separation plants. No significant differences in male hormone production by testis tissue slices exposed to WAF, or in the expression of genes associated with reproductive or thyroid signaling were recorded. However, the expression of the polycyclic aromatic hydrocarbon (PAH) sensitive cytochrome P450 1a1 was significantly upregulated in response to WAF exposure. Gene expression biomarkers in Mozambique tilapia exposed for a short period to WAF indicated a risk of disruption of the male and thyroid hormonal systems, but only at a high WAF concentration. A longer term, premetamorphic tadpole exposure, showed that the WAF has the potential to disrupt the thyroid systems of exposed wildlife. In addition, the aromatase coding, cyp19a1 gene was significantly altered. However, none of the other 15 genes evaluated, representing multiple pathways, were significantly modulated following WAF exposure. In general, our data suggest a low risk for freshwater aquatic vertebrates, exposed to aged crude oil form this old underground bunker or in the downstream sites of the receiving waters.

390 Respirometry and Swim Performance Alterations in Zebrafish (Danio rerio) acutely exposed to Hydraulic Fracturing Flowback and Produced Water

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Horizontal hydraulic fracturing is an emerging practice in North America to extract oil and gas reserves. To date, little toxicological information is supplied for both the technologies and chemicals used during the fracturing process, and for the surface returned by-product fracturing flowback and produced water (FPW). Volumes of water used per well can often exceed 1 million m^3, resulting in potentially devastating environmental effects when spills of FPW occur. Although salinity dominates much of the toxicity observed, other inorganic (e.g. metals, radionuclides, etc) and organic (e.g. polyaromatic hydrocarbons, benzenes, etc.) molecules found in FPW can induce toxicological responses in exposed organisms. The current study uses zebrafish (Danio rerio) as a model organism to determine if acute exposures to FPW induce developmental changes in organismal oxygen uptake. To determine if exposure to dilute FPW resulted in changes in oxygen consumption, zebrafish embryos were acutely exposed to 2.5% and 5% FPW and oxygen consumption was measured. Transcriptional analyses of specific hypoxia, stress inducible, and cardiovascular genes were performed and paired with embryo metabolic rates to determine if FPW exposure affected immediate embryo oxygen consumption, and if FPW-induced respirometry changes persisted after exposure was terminated. Finally, juvenile zebrafish swim performance experiments were performed to determine if acute FPW exposure affected later stage fish fitness. This is one of the first studies to use respirometry as a metric for measuring FPW induced toxicity. Our results aim to validate respirometry as a potential toxicological biomarker for identifying biological zones of impact when FPW spills occur, and provide regulating agencies information to help shape future FPW spill/leak remediation protocols.

391 Rapid adaptation to oil exposure in the cosmopolitan copepod Acartia tonsa

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Oil spills are potential environmental hazards to marine ecosystems worldwide. Oil spills may persist in seawater longer than one generation of many zooplankton species. However, whether populations of short-lived and fast growing marine organisms adapt to oil exposure through natural selection is not known. To test this, the cosmopolitan estuarine copepod Acartia tonsa was exposed to pyrene continuously for two generations, at the concentrations 0, acetone control, 1, 10, 100 and the saturated pyrene concentration in seawater, 100+ nM. Pyrene is one of the most toxic components in crude oil to marine copepods. The key fitness-related traits were quantified: survival, size at maturity, grazing rate and the reproductive success. Exposure to the concentration of pyrene saturated in seawater (100+ nM) resulted in 100 % mortality before adulthood in the first generation. In the other treatments (≤ 100nM), the first generation had a higher grazing rate than the second generation. Exposure to pyrene had no effect on the grazing rate. At the concentration of 100 nM, pyrene exposure caused reductions in survival, size at maturity of females, egg production and hatching success. The reduction in size at maturity of females was less pronounced in the second generation. Strikingly, both survival, egg production and hatching success were recovered in the second generation, indicating a rapid selection towards individuals with adaptations to deal with pyrene exposure. Our results show that populations of short-lived and fast-growing copepods have the potential of showing surprisingly strong resilience to the type of oil contamination they might face in their natural coastal habitats. *Joint first authors: Kamille Elvstrøm Krause and Khuong Van Dinh

EDCs and Pharmaceuticals in the Environment – Part 1

392 Analysis on the Effects of Pharmaceuticals and Their Uptake and Metabolism in Plants Using Liquid Chromatography Tandem Mass Spectrometry

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There has been recent interest in agriculture to recover and reuse nutrients in a renewable way. Specifically efforts have been contrived to replace typical chemical fertilizers with something more sustainable and recyclable from the environment including urine and wastewater. Both of these prospective fertilizers are known to contain pharmaceuticals and application of these to farm lands may further the release of biological contaminates into the environment. Chlorinated pharmaceuticals are of particular concern due to their potential for greater toxicity, specifically in regards to human health and exposure from chlorinated pharmaceuticals uptaken food crops. A study we have done on the analysis of lettuce and carrots exposed to pharmaceuticals by liquid chromatography tandem mass spectrometry (LC-MS) show that they do uptake both the chlorinated and unchlorinated pharmaceuticals. While all pharmaceuticals, including the chlorinated ones (diclofenac and sulfachloropyrazidine) may be uptaken in their native form, studies have shown that certain crops metabolize chlorinated pharmaceuticals and detoxify them. Our study focuses on the fate of pharmaceuticals in corn grown hydroponically. The same pharmaceuticals in the study with lettuce and carrots were used in the exposure with corn. The results showed that all the pharmaceuticals were uptaken except the chlorinated ones, suggesting some transformation or metabolism of these pharmaceuticals took place. The hypothesis is that glutathione transferase is catalyzing the removal of chlorine and conjugation of the pharmaceutical to the sulfur on the glutathione. This phenomena is investigated by LC-MS by first extracting the glutathione transferase from the plant (using affinity chromatography and confirmation by SDS-PAGE) and doing an in vitro study with the transferase, pharmaceutical, and glutathione. From there, the conjugated pharmaceutical is monitored for in the plant extract. This research is important for understanding the fate of pharmaceuticals in corn which is of utmost importance when considering the effects of using pharmaceutical containing fertilizers for agricultural use.

393 The fate of antibiotics and antimicrobial biocides in Swedish sewage treatment plants

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Besides antibiotics, a large number of antimicrobial biocides, used as preservatives, disinfectants, and antiseptics, are entering our sewage system. There is a concern that biocides promote the development of antibiotic resistant bacteria through the mechanisms of co- and cross resistance. It is therefore important to measure the presence of these substances in sewage water and sludge in order to better understand their fate and transport in the environment. To address these issues, an analytical protocol using liquid chromatography tandem mass spectrometry (LC-MS/MS) was developed for measuring biocides and antibiotics in sewage sludge and waters. Further, 11 Swedish sewage treatment plants (STPs) were sampled for influent, effluent, and digested sludge during three consecutive days. In addition, three of the STPs were subjected to an extensive nine-day sampling campaign during three weeks including samples from various types of sludge, and water after the first sedimentation step. In total, 246 samples were collected for determination of their content of antimicrobial biocides and antibiotics. Several compounds could be detected in the different sample matrices including various quaternary ammonium compounds (QACs), isothiazolines, chlorhexidine, antibiotics, antimycotics and benzotriazoles. Mass flows could be calculated for many compounds and QACs, e.g. didecyldimethyl ammonium chloride (DDMAC), cetrimonium bromide (CTAB), and benzalkonium chloride, were the most abundant ones with levels of several kilograms/day in incoming waters (aqueous + solids). The outgoing water mass balances of QACs showed only a few grams and the majority of these compounds end up in the sludge where they could be detected in levels up to 200 µg/g d.w. The highest levels in outgoing water effluents were the benzotriazoles with levels exceeding 1 µg/L and mass balances of 100 g/day in some STPs. Interestingly, chlorhexidine was not only detected in high levels but also frequently in sludge of all studied STPs, results that stand in contrast to previously published studies. Common detected antibiotics were ciprofloxacin, tetracycline and trimethoprim. Knowledge of fate and occurrence of biocides within STPs are important to aid in risk assessments of the antibiotic resistance development and this work demonstrates a comprehensive data set for Swedish STPs.

394 Occurrence of contaminants of emerging concern (CECs) in reclaimed water intended for potential potable reuse

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Due to the drought and water scarcity in some parts of the country, there is an increased attention to the sustainable water management. Wastewater effluent is a viable alternate option as source water. However, there are potential public health risks associated with exposure to the microbial and chemical contaminants present in the wastewater effluents. Additional research is needed to further characterize the microbial pathogens and contaminants of emerging concern (CECs) present in wastewater effluent and to evaluate overall efficacy of the advanced treatment processes used to further disinfect and treat the effluent. To better address these challenges, EPA ORD and EPA Region 6 have partnered on a research project focused on waterborne pathogens and CECs that are present in wastewater effluent from an advanced wastewater treatment plant and racetrack plants. The on-going research project characterizes microbial communities and occurrence and composition of selected CECs in wastewater effluent that will serve as source water to water reuse plants. The current presentation focuses on the concentration trends of selected CECs. Several classes of contaminants are monitored, such as poly and perfluoroalkylated substances (PFASs), steroid hormones, alkylphenol ethoxylates, alkylphenols, pharmaceuticals and personal care products. During a period of one year, monthly grab samples of influents and effluents are collected from 3 publicly Owned Treatment Works (POTW) in Region 6. The treatment processes used by these POTWs include chlorination, UV disinfection, and microfiltration, reverse osmosis followed by UV oxidation. The samples are extracted separately for different classes of CECs and are analyzed on UPLC/MS/MS. Based on the concentrations and compositions of CECs, seasonal effects and differences in the treatment processes will be evaluated. The data on the occurrence and concentrations of different CECs and potential impacts (e.g. increase in levels of contaminants) of using wastewater effluent as a drinking water source on the overall water quality of the drinking water produced will be discussed. This project will assist the States and Regions that use wastewater effluent as a drinking water source by providing data on the occurrence and persistence of potential waterborne pathogens and CECs.

395 Distribution of Endocrine Disrupting Compounds in the Aquatic Food Web, Lake Mead, Arizona and Nevada

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Water quality in Lake Mead, Arizona and Nevada, is affected by contaminants coming from the Las Vegas Wash, an urban perennial stream whose water is comprised almost entirely of treated wastewater effluent coming from the Las Vegas metropolitan area. Endocrine disruption, presumably resulting from the uptake and/or accumulation of contaminants, has already been demonstrated in common carp from Las Vegas Wash and both common carp and largemouth bass in Las Vegas Bay of Lake Mead. This study examined how various endocrine disrupting compounds (EDCs) are distributed in organisms throughout the food web in Lake Mead. Three areas of the lake were examined; Las Vegas Bay, Boulder Basin, and Overton Arm. Las Vegas Bay is the basin which receives contaminants from Las Vegas Wash while Overton Arm is farthest from Las Vegas Wash and represented a control site. Boulder Basin represented an intermediate position betwen these two. To determine water concentrations of contaminants, SPMDs and POCIS passive samplers were deployed at each site. Samples of periphyton, zooplankton, benthic invertebrates, quagga mussels, and a representative mix of forage fish were collected and analyzed for stable isotopes of carbon and nitrogen in order to determine trophic position and for over 200 organic compounds that have the potential to be EDCs. Some compounds show increasing concentrations with increasing trophic level while others do not. For some compounds sampled by POCIS, concentrations by trophic level relative to concentrations in water indicate potential accumulation from water. When examined by compound group, the slope of concentrations versus trophic level is higher in biota from Las Vegas Bay relative to biota from either Boulder Basin or Overton Arm indicating that accumulation of contaminants is greatest near the source. Although the concentrations of some compounds increased in the food web, the specific endocrine-disrupting effects of most are not clear. Since some compounds are estrogenic, a Yeast Estrogen Extract test was performed on water extracts from each site. Estrogen Equivalents were detected at 2 sites in Las Vegas Bay (0.22 pg/L and 0.08 pg/L) but were not detected in Boulder Basin and Overton Arm. These results suggest that contaminants with estrogenic activity, and thus possible endocrine-disrupting effects, are most closely associated with wastewater coming from Las Vegas Wash.

396 Mass loading, removal and environmental emission of select psychoactives, antihypertensives, and antibiotics from two sewage treatment plants in India

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Environmental contamination by pharmaceuticals and personal care products (PPCPs) is barely conceived in India despite being one of the largest global producers and consumers of pharmaceuticals. In this study, 29 pharmaceuticals and six metabolites were determined in a STP in Udupi (STP_U: population served ~150,000) and a STP in Mangalore (STP_M: population served ~450,000); the total concentrations were 99.5 µg/L and 51.8 µg/L, respectively. Atorvastatin (the most prescribed antihypercholesterolemic in India), mefenamic acid, and paraxanthine were found for the first time in wastewater in India at the mean concentrations of 395 ng/L, 1100 ng/L, and 13,000 ng/L, respectively. Select pharmaceutical metabolites (norverapamil and clopidogrel carboxylic acid) were found at concentrations of upto 7 times higher than their parent drugs in wastewater influent and effluent. This is the first study in India to report mass loading and emission of PPCPs and their select metabolites in STPs. The total mass load of all PPCPs analyzed in this study at STP_U (4.97 g/d/1000 inhabitants) was 3.6 times higher than calculated for STP_M. Select recalcitrant PPCPs such as carbamazepine, diazepam, and clopidogrel were found to have negative or no removal from STP_U while additional anaerobic treatment with upflow anaerobic sludge blanket reactor at STP_M removed (up to 95%) these PPCPs from STP_M. Overall, 5.1 kg of caffeine, 4.1 kg of atenolol, 2.7 g of ibuprofen, and 1.9 kg of triclocarban were discharged annually from STP_U. The PPCP contamination profile in the Indian STP was compared with a similar study in the USA.

397 High-throughput Based Screening of Waters for Endocrine Bioactive Chemicals

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The presence of industrial chemicals and pharmaceuticals is well documented in wastewater, surface waters, and to a lesser extent in drinking water. Many of these chemicals lack human health-protective guidelines, but many have been shown to have endocrine bioactivity and thus the potential for endocrine disruption. We present an approach for evaluating potential endocrine activity of chemicals detected in drinking water based on in vitro high throughput screening assays and predictive pathway models from the USEPA's ToxCast program. ToxCast bioactivity data for estrogen receptor (ER) and androgen receptor (AR) signaling pathways are currently available for over 1800 chemicals. The toxicokinetic data necessary for in vitro to in vivo extrapolation of dose response are available for a subset of 90 of these chemicals with ER or AR bioactivity; 47% are pesticides and 27% are pharmaceuticals. For each of these 90 chemicals, high throughput data were used to generate Ambient Water Bioactivity Concentrations (AWBC): water screening concentrations analogous to Ambient Water Quality Criteria for the protection of human health. A range of AWBCs for each chemical were calculated based on minimum and median ER or AR bioactivities across ToxCast assays, high-throughput toxicokinetic data, and data driven assumptions about consumption of water. For chemicals that are both ER and AR bioactive, AWBCs were calculated for both pathways and the lowest value in either pathway was selected. Using a 2015 monitoring dataset of waters in metropolitan Denver, measured concentrations of pesticides, steroids and waste indicators were compared with their chemical-specific AWBCs. The reporting limits and the maximum measured concentrations for 17 beta-Estradiol, 17alpha-Ethylnylestradiol and estrone exceed the lowest calculated AWBC variant. Triclosan was within one order of magnitude, and bisphenol A and oxazepam are within three orders of magnitude of their respective AWBCs. The results are dominated by measurements of estrogenic substances and the lack of water monitoring for androgenic chemicals. Cumulative endocrine activity of chemical mixtures contained in each sample was also evaluated using a hazard index-like approach. This analysis demonstrates an approach to screening for potential endocrine activity in drinking water and could inform prioritization of future monitoring, testing and pollution prevention efforts. This abstract does not necessarily reflect EPA policy.

398 Characterization of the endocrine potencies of municipal effluents across Canada using in vitro bioassays

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Recent years have witnessed increasing concerns regarding the presence of contaminants in the environment that have the potential to adversely affect the endocrine system of humans and wildlife. Municipal wastewater effluents (MWWEs) are considered one of the major sources for such endocrine disrupting chemicals (EDCs) in surface waters, as conventional wastewater treatment technologies are frequently inefficient at removing these compounds from raw sewage. However, our understanding of the contribution of MWWEs to environmental endocrine disruption in Canadian surface waters is incomplete. Therefore, the aim of this project was to investigate the EDC removal efficiency of six wastewater treatment plants (WWTPs) across Canada. Specifically, samples of influents and effluents were collected during spring, summer and fall in 2014 and winter in 2015, to evaluate the influence of climatic conditions, season, population size and treatment level (tertiary, secondary, primary) on EDC removal efficiency. Endocrine potentials of wastewater were analyzed using two in vitrobioassays: MDAkb2 ((anti-)androgenicity), and MVLN ((anti-)estrogenicity). Preliminary results indicated that most influent samples collected had a significant increase in androgenicity relative to solvent controls, while most effluent samples were less potent and showed variability in response indicating that WWTPs had a high removal efficiency of androgenic activity. Removal efficiencies differed significantly among WWTPs, probably as a function of different levels of treatment. Greater removal efficiency was detected during summer, potentially due to greater metabolic activity, increased temperature and/or light exposure. A select number of influent and effluent samples showed anti-androgen response, suggesting there may be other compounds competing for the same receptor. Most spring effluent samples showed a trend towards elevated estrogenicity, as did fall samples from Regina and Saskatoon. Effluent samples showed higher estrogenic activity than influent samples, and anti-estrogenic trends were detected for the majority of influents and effluents, suggesting samples may contain compounds competing for the same receptor. This project will provide insights into the most effective approach for monitoring MWWEs, and will inform development of advanced wastewater treatment technologies for improved removal of EDCs.

399 Examining Urban Metabolism and Contaminants of Emerging Concern: A Sewage Epidemiology Pilot Study in Hong Kong

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High concentration of human populations is readily observed in Hong Kong, which now represents the 4th most densely populated area in the world. Such population densities result in concentrated use of specialty and industrial chemicals, including pharmaceuticals and consumer products. Much like a watershed in the natural world includes a network of smaller streams flowing to larger river systems and ultimately coastlines, waste streams in urban areas are collected by diverse sewerage systems from residential, commercial and industrial users. In fact, different component of wastewater collection systems represent "sewersheds" because they are conceptual similar geospatial units to natural watersheds and thus can be characterized by differential fingerprints of urban metabolism. Whether pharmaceutical or elicit drug consumption differs among different areas of Hong Kong is not understood. In this pilot study, we focused on the wastewater primary treatment works (PTWs) in the Kowloon peninsular and north-east Hong Kong Island. Specifically, eight PTWs collect wastewater from different sewersheds, which collectively covers a significant proportion of the urban population in Hong Kong, and transports effluents to Stonecutter Island (SCI) Treatment Works. We sampled each of these sewersheds and the SCI influent and effluent to determine whether target analytes, including common pharmaceuticals, drugs of abuse and other anthropogenic tracers, and associated therapeutic hazards differed among various sewersheds. Our sampling effort accounted for travel times among each of the PTWs to SCI, based on previous tracer studies, which allowed us to apportion drug waste contributions among the 8 PTWs to SCI. From each sampling point, water analytes were extracted, separated and analyzed following previously reported methods using solid phase extraction and isotope dilution-liquid chromatography-tandem mass spectrometry. We then used a fish plasma modelling approach to determine whether water quality hazards differed among each PTW contribution to SCI. Consistent with reports from previous efforts, highest concentrations were consistently observed for caffeine, acetaminophen and the artificial sweetener sucralose. However, marked differences were observed among analytes and associated therapeutic hazards across various sewersheds examined in Hong Kong. These results may inform decisions regarding waste stream assessment and management in specific regions.

Fate, Toxicology, or Risk Assessment of Materials of Interest to the Military

400 Ecotoxicological risk of explosives and heavy metals on aquatic species in surrounding water system nearby active firing range

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Explosives contamination at firing ranges is known to have impact on the surrounding ecosystem. In addition, contaminants can be discharged to the neadby river due to precipitation. It is more reasonable to manage the contaminants in the runoff, since active soil remediation is impractical for most of highly active firing ranges. This study was performed to determine the ecotoxicological effects of explosives and heavy metal in the surrounding water system. Among explosives and heavy metals, 2,4,6-trinitrotoluene (TNT), hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX), lead (Pb), copper (Cu), cadmium (Cd), and zinc (Zn) were selected as target pollutants. Toxicological information of each contaminants was collected from ECOTOX of US Environment Protection Agency (EPA) and additional technical papers. Chronic no observable effect concentration (NOEC) and effective concentration (EC10) data were chosen and subsequently toxicity data without clear statement of experimental conditions were screened out in the screening procedure. Species Sensitivity Distribution (SSD) was used for the derivation of hazardous concentration (HC5, HC50) of each pollutants. Predicted no effect concentration (PNEC) was calculated and suggested as 40 – 88, 270 – 600, 1.9 – 9.4, 0.23 – 1.1, 0.19 – 0.96, 1.6 – 7.8 µg/L for TNT, RDX, Pb, Cu, Cd, Zn, respectively using uncertainty factor from 1 to 5. Hazard quotient (HQ) was derived using PNEC values and predicted environmental concentration (PEC) of active firing range in Korea. HQ values of TNT, RDX, Pb, Cu, Cd, Zn were calculated as 14.5 – 32.0, 0.78 – 0.35, 53.2 – 263.2, 45.5 – 217.4, 52.1 – 263.2, 56.2 – 273.8 indicating TNT, Pb, Cu, Cd, Zn have toxicological effect on aguatic species in nearby water system. This ecotoxicological study will be used to quantify the toxicological risk of explosives and heavy metals to the surrounding ecosystem after the remediation process.

401 Toxicological Effects of Munitions Compounds and their Breakdown Products on Coral

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Military activities in marine environments have resulted in munitions and unexploded ordnance being located throuout the world's oceans, yet the ecological impacts to marine life from the associated chemical constituents and their breakdown products remains largely unknown. Coral reefs are particularly vulnerable ecosystems found in proximity to many key U.S. military installations in the Caribbean and Pacific Islands. We investigated whether munitions compounds or their breakdown products impact coral health by conducting standard laboratory toxicity testing of nine munitions compounds (TNT, 2,4-DNT, 2,6-DNT, 2,3-DNT, 4-NT, 2-ADNT, RDX, HMX and picric acid) with Pocillopora damicornis coral. NOEC and LOEC values and effect-concentration (LC or EC) values were established using a coral cell toxicity assay. Effects of these compounds on the coral symbiont were also tested using Symbiodinium sp. in vitro cultures with photosynthetic efficiency and mortality as endpoints. Findings showed all nine munitions coumpounds had some level of toxicity, differences in coral species' sensitivity to munitions compounds, and photo-enhanced toxicity of certain compounds. The toxicity reference values for corals exposed to munitions compounds will provide metrics for assessing risk of munitions to coral and coral reef health while serving as a basis for establishing screening and action values for management of this vulnerable resource.

402 Aquatic Toxicity of the Insensitive Explosive DEMN

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DEMN is an insensitive energetic mixture that consists of a eutectic mixture of diethylenetriamine trinitrate (DETN), ethylenediamine dinitrate (EDDN), methyl nitroguanidine (MeNQ), and nitroguanidine (NQ). It was developed by the Army Research Laboratory as a melt-cast explosive to replace trinitrotoluene (TNT), an explosive that can be sensitive to unintentional detonation and also known to negatively impact occupational health. Because the DEMN components are relatively soluble, data on their aquatic toxicity were needed to determine potential environmental impacts. All five elements of the composition were tested—the DEMN eutectic mix and each of the four components. The first round of tests consisted of acute range-finding tests with Daphnia, fathead minnow, and green algae. With the exception of the Daphnia values for the DEMN eutectic, DETN, and EDDN, all of the values obtained for the test systems exceeded Globalized Harmony System (GHS) Acute Aquatic Toxicity Category 3 (>100 mg/L), making them essentially non-toxic in an acute exposure scenario; the remaining values were between one and ten mg/L, which is GHS Acute Aquatic Toxicity Category 2. These initial tests were completed to help determine exposure levels for the chronic exposure tests. The chronic exposure tests included the Daphnia acute, fathead minnow acute, fathead minnow embryo-larval survival and teratogenicity, Ceriodaphnia survival and reproduction, and green algae growth toxicity tests. Only the DEMN eutectic, EDDN, and DETN were evaluated in this second round of tests due to the results of the initial acute toxicity evaluations and the chemicals' water solubility. All three of these substances were ranked as GHS Chronic Aquatic Toxicity Category 3 (LC_{50} >10 but < 100 mg/L) for the Daphnia acute, green algae, and Ceriodaphnia chronic tests, and Category 4 (LC_{50} >100 mg/L or above) for the fathead minnow acute and chronic tests. Chronic and multi-generation toxicity tests report adverse effects to TNT in fathead minnows at exposure levels as low as 0.24 and 0.04 mg/L, respectively. The data reported here suggest that DEMN and its constituents have a low potential for toxicity to aquatic organisms.

403 Birds are not feathered mammals: phylogenetic differences in toxicity from oral exposures to 3-Nitro-1,2,4-Triazol-5-One (NTO)

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Insensitive munitions (IM) are currently being developed to protect against unintentional detonation in theaters of operation. Testing and training with these materials at military ranges are expected to result in environmental releases which may constitute a risk to valued wildlife species. Acute oral studies conducted in rats did not result in overt toxicity from exposures exceeding 2000 mg/kg and subchronic oral exposures found reductions in testes and epididymides mass, as well as oligospermia, at 315 mg/kg-d but not at 100 mg/kg-d. An extended one-generation study conducted in rats from exposures to NTO in drinking water found testicular changes and delays in puberty for male offspring at exposures of 3600 mg/L (157-335 mg/kg-d). To investigate conservation of testicular toxicity among vertebrates, an extended one-generation reproductive toxicity test was conducted with Japanese quail (Coturnix japonica) where both sexes of the parental generation were exposed to NTO at 0, 20, 100, 500, and 1000 mg/kg-d via oral gavage beginning at two weeks of age. First generation (F1) birds were exposed to NTO via oral gavage beginning at day 2 of age. All birds were dosed for 10 weeks. Following 5 days of treatment, F0 birds from the 1000 mg/kg-d group exhibited neuromuscular toxicity (convulsions and ataxic wing activity) 3-4 hours post initial exposure. Birds from the 500 mg/kg-d group began displaying similar signs 17 days into treatment. In both dose groups, convulsions initially ceased after 3-4 hours. No birds from the 1000 mg/kg-d group survived and only a single male from the 500 mg/kg-d group survived until study completion. No changes in testes mass or sperm production were found in birds from the 20 and 100 mg/kg-d groups, though the single surviving male had a reduction in testes mass compared to males from the control, 20 and 100 mg/kg-d groups. Preliminary analysis show changes in electrolyte levels which may indicate nephrotoxicity of NTO specific to birds.

404 Geographical distribution of contaminants in the soil at a Canadian military test site

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In the context of an ecological risk assessment (ERA) that is being conducted at a military test site in eastern Canada, data on soil quality collected over many years from the various areas on the site were pooled in order to assess the extent of the contamination. A sampling plan was then prepared in order to fill gaps in the data. This included measuring the concentration of potential contaminants of concern that had not been previously analyzed near active or historical sources. It also included the measurement of contaminants around known contaminated sites in order to circumscribe the extent of the contamination. This was particularly important from an ecotoxicological point of view, as there was little information on the level of contamination in the natural habitats surrounding the active ranges found on the site. The results indicated that some substances, such as 2,4,6-trinitrotoluene (TNT) as well as many other military specific compounds, are only found at levels above the ecological screening values at the center of the active test sites. These substances are therefore unlikely to be a source of risk to ecological receptors, as they are not found within the habitats used by these species. In other cases, notably aluminum, all measurements exceed the screening levels, indicating a high likelihood that the natural background of the site is higher than the ESL. Further analysis is therefore required to determine if the current and historical activities on the site constitute a significant source of this contaminant and to determine the extent of the contamination.

405 Verification Monitoring and Stability Assessment of In Situ Stabilized Range Metals

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Where appropriate, several measures of long-term remedy effectiveness and technology performance should be evaluated at demonstration sites in addition to the key measure of long-term risk reduction. A major thrust of this effort is to validate best management practices for metals stabilization with phosphate amendments and answer questions regarding the long term stability of the approach under varying environmental conditions. The current study assesses the post remedial, long-term effectiveness of a field demonstration design on the M-60 range at Charleston Air Force Base (AFB) in Charleston, South Carolina. The Charleston AFB testing includes a 5% TRAPPS™ amendment, alone and in combination with, 5% fish bone based apatite for in situ stabilization of lead in berm soil. Soil samples have been collected and analyzed for metals, pH, soil fractionation, speciation, and other leaching tests by standard USEPA and ASTM methods. Findings from this effort are presented to evaluate long-term effectiveness and monitor the stability of Pb present at the demonstration site.

406 Exposure to munition specific carcinogens and cancer risks for civilians on Vieques following military exercises '47-'99

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The total loading of carcinogens to the island of Vieques has been documented by reviewing the records from to the U.S. National Archives on munitions used in testing and exercises on the island from 1947 to 2003. With these data, the exposure pathways (air; water; soil; food) specific to humans were modelled. Subsequently, the added cancer risks and Margins of Exposure (MoEs) for the different carcinogens for each year were derived. The results show that there was a potential for cancer risk concern with regards to RDX exposures in the 52 years of military activity due to a cancer risk of $5*10^{-5}$ in the general population. Furthermore, there was a potential for concern of health risks from TNT exposures with a MoE of 48. No other exposures indicate a concern for risk. Both of these exposures primary exposure route was oral via produce leafs, fruits and cereals. There is in general no significant difference in the cancer incidence rates between Vieques and Puerto Rico. However, the period 1992-1997 did show a significantly elevated lung and bronchus cancer incidence rate in Vieques compared to Puerto Rico mainly among women < 50 yr and men 50-64 yr, which correlate with exposures in the period 1977-1984. Both cancer and other public health impacts and risks are multi-causal, hence, the general stress impacts due to military training on Vieques towards public health of Viequenses, would warrant a wider community-based public health and risk assessment assessing past, current, future potential epigenetic, risks.

407 Mass Flux Characterization for Determination of Reasonable Maximum Exposures to Volatile Organic Compounds through the Vapor Intrusion Pathway

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Vapor intrusion (VI) is challenging to assess using conventional discrete indoor air and sub-slab sampling because of spatial and temporal variability in volatile organic compound (VOC) concentrations, which leads to uncertainty in identifying a Reasonable Maximum Exposure (RME) for the occupants of a building. Characterization of vapor phase contaminant mass flux (mass per unit time per unit area) offers a promising alternative to conventional indoor air and sub-slab sampling because mass flux measurements have been found to exhibit considerably less temporal variability than indoor air measurements. Several methods of mass flux characterization are being studied as part of an ESTCP SERDP demonstration program (ER-201503), including measuring the mass flux through a building during "forced" depressurized conditions that enhance vapor transport into the building. The indoor air concentrations measured during building depressurization provide upper bound estimates of the indoor air concentrations that may arise from vapor intrusion under RME conditions. Additionally, the relative contribution of background (indoor) vapor sources to indoor air concentrations can potentially be identified by pressurizing the building to hinder or reverse vapor intrusion; any detected VOCs in indoor air under that condition necessarily arise from ambient or indoor sources. The combination of building depressurization and pressurization is referred to as Building Pressure Cycling (BPC). BPC can be conducted in a relatively short timeframe (e.g., 2 -12 hours) to characterize VI pathway impacts regardless of the season or climatic conditions. This paper describes the protocol used to conduct BPC to defensibly identify RME concentrations and compares the results from field application of BPC to historical VI characterization data. These results suggest that mass flux characterization may be a more effective and less expensive than VI site characterization using multiple conventional sampling events.

Toxicity Extrapolations in Aquatic Organisms and Wildlife

408 Rethinking the Use of Uncertainty Factors for the Derivation of Toxicity Reference Values

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Uncertainty factors (UFs) are commonly used to account for multiple sources of uncertainty in the derivation of toxicity reference values in ecological risk assessment. Common examples include estimating the dose range between a lowest-observed-adverse-effect level (LOAEL) and a no-observed-adverse-effect level (NOAEL), extrapolating doses associated with chronic effects from doses associated with subchronic or acute effects, accounting for interspecies variability, and accounting for differences in laboratory and field conditions. While it is convenient to apply the simplifying assumption that UFs of unit values (e.g., 3, 5, 10) are sufficiently protective, it is important to revisit these assumptions as new studies and methods for extrapolating toxicity data become available. In this presentation, we revisit the basis for applying UFs to extrapolate between LOAELs and NOAELs and demonstrate how the use of dose-response relationships in ecological risk assessments can provide an alternative and more scientifically defensible representation of effect ranges between study dose levels. Using published toxicity data on methyl mercury effects in birds, we explore several ways to use dose-response information in lieu of NOAEL/LOAEL ratios and discuss data needed to extend the methods across chemicals and receptor groups.

409 The chemistry side of AOP: implications for toxicity extrapolation

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An adverse outcome pathway (AOP) is a structured representation of the biological events that lead to adverse impacts following a molecular initiating event caused by chemical interaction with a macromolecule. AOPs have been proposed to facilitate toxicity extrapolation across species through understanding of species similarity in the sequence of molecular, cellular, organ and organismal level responses. However, AOPs are nonspecific regarding the identity of the chemical initiators, and the range of structures for which an AOP is considered applicable has generally been poorly defined. Applicability domain has been widely understood in the field of QSAR as the response and chemical structure space in which the model makes predictions with a given reliability, and has been traditionally applied to define the similarity of query molecules within the training set. Three dimensional (3D) receptor modeling offers an approach to better define the applicability domain for selected AOPs through determination of the chemical space of the molecular initiating event. Universal 3D-QSAR models were developed for acetylcholinesterase inhibitors and estrogen receptor agonists and antagonists using a combination of fingerprint, molecular docking and structure-based pharmacophore approaches. The models were based on the critical molecular interactions within each receptor ligand binding domain, and included the key amino acid residues responsible for high binding affinity. The approach allows for extrapolating species sensitivity based on taxa-specific differences in these key amino acids and estimating toxicity for a broad range of chemical structures. Continuing improvements in conformer generation and molecular docking tools, and increasing availability of x-ray crystal structures of receptor-ligand complexes will allow for more efficient and accurate 3D-QSARs and advancement of AOPs from organizing frameworks to predictive models.

410 Integrating Aquatic Interspecies Toxicity Estimates into Large Databases: Model Evaluations and Data Gains

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The Chemical Aquatic Fate and Effects (CAFE) database, developed by NOAA's Emergency Response Division (ERD), is a centralized data repository that allows for unrestricted access to fate and effects data. While this database was originally designed to help support decisions in the event of spill incidents in aquatic environments, this data repository could help address a diverse range of research and response needs. One of CAFE's features is that it contains aquatic toxicity data for several thousand chemicals allowing for the visualization of data in the form of probabilistic distributions of toxicity data across taxa or Species Sensitivity Distributions (SSDs). While CAFE has enough data to generate over 2,000 chemical-specific SSDs, 70% of compounds including hazardous substances with high spill risk have only limited data (< 5 species). These data gaps may be filled by integrating into CAFE Interspecies Correlation Estimation (ICE) models, which are log-linear regressions between a surrogate species and a predicted taxa. This integration required a multistep process to ensure data quality and identify the best available models. Evaluations of over 3,500 ICE models included the influence on SSD estimates from models that: 1) did not meet three reliability requirements (i.e., Mean Square Errors < 0.95, slope >0.6, adjusted coefficients of determination [adj-R^2] >0.6); and 2) were originally developed based on broad and specific mode of toxic action (MOA), or from combined MOA models. Preliminary evaluations of ICE-predicted toxicity data for a variety of chemicals and surrogate species suggest that in most cases (76%) the exclusion of those less reliable models does not lead to substantial changes in SSD estimates, which were generally within a 3-fold of values obtained with models meeting all reliability requirements. Although MOA appears to have a greater influence on SSD estimates than model reliability, MOA is uncertain for many chemicals, thus limiting their use. The most significant contribution of this work is that the integration of ICE models into CAFE would allow for the development of SSDs for least 67% of 4,800 total chemicals with limited aquatic toxicity data, while increasing the number of SSDs by 30%. Most data gains are for standard exposure durations (24, 48, 72 and 96 hours), which comprise 85% of data used to generate ICE-based estimates. The integration of ICE models may substantially enhance the current capabilities of CAFE.

411 Effects of Deepwater Horizon Oil on Red-Eared Sliders and Common Snapping Turtles as Surrogate Species for Sea Turtles

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Hundreds of externally and internally oiled juvenile oceanic sea turtles were documented and rescued during the Deepwater Horizon (DWH) oil spill. Polycyclic aromatic hydrocarbon (PAH) metabolites in the bile and tissues of some individuals confirmed internal exposure and metabolism of oil. The adverse effects associated with becoming physically mired in oil were readily apparent in sea turtles rescued during the DWH spill, but data on potential effects of oil ingestion in sea turtles are limited. Therefore, a 14-day, controlled-dose ingestion laboratory study was undertaken using DWH oil (MC 252) to examine potential effects of internal oil exposure on common species of freshwater turtles, red-eared sliders and common snapping turtles as surrogates for endangered sea turtles. Numerous chemical, biological, and physiological endpoints were assessed, including those important for general clinical assessment, as well as additional endpoints known to respond to acute exposure to PAHs in other taxa (e.g., oxidative stress, DNA damage). Specifically, effects on the hypothalamic-pituitary-adrenal (HPA) axis, which regulates stress response and other vital functions, were investigated due to observations of potential HPA disruption in other vertebrate species exposed to DWH and other crude oil. We will present results from the full suite of physiological and toxicological endpoints examined in this study, as well as the implications for the DWH sea turtle injury assessment and for future assessments of toxic effects of oil to turtles.

412 Mercury Accumulation and Effects in the Brain of Atlantic Sharpnose Sharks (Rhizoprionodon terraenovae)

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Sharks often bioaccumulate mercury (Hg) concentrations in their muscle to levels that threaten the health of human consumers. However, few published studies have examined if the high Hg levels seen in shark muscle also occur in the shark brain, or if Hg accumulation affects shark neurophysiology. Therefore, this study examined if shark brains accumulate significant levels of Hg, if Hg accumulation occurs in certain subcomponents of the brain, and if Hg accumulation is associated with effects on the shark central nervous system, with special focus on the Atlantic sharpnose shark (Rhizoprionodon terraenovae). Sharks were collected along the U.S. Southeastern coast. Muscle and brain Hg concentrations in the sharks were determined by the Direct Mercury Analyzer. Correlations were examined between brain Hg concentrations and levels of known biomarkers of Hg-induced neurological effects (e.g., levels of a protein biomarker of glial cell damage, S100b, and markers of oxidative stress) in shark cerebrospinal fluid (CSF). Brain Hg levels were correlated with muscle Hg levels, but were significantly lower and did not exceed most known thresholds for neurological effects, suggesting limited potential for such responses. Data on CSF biomarker levels support this premise, because they were not correlated with brain Hg levels. Higher Hg levels were measured in the forebrain of shark in comparison with the hindbrain, but levels in both were below threshold levels for effects. This study is the first to demonstrate the correlation and significant difference of Hg in the brain and muscle of sharks. It is also the first to identify significantly higher Hg levels in the forebrain. This study is the most extensive analysis of Hg in a single shark species, spanning most of its Atlantic range. It is also the first to examine neurological effects of Hg exposure in these animals.

413 Phylogenetic Signal in Fish Acute Toxicity

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Traditionally, risk assessment has focused on a few model test species to represent toxicity of chemicals to ecological receptors. Recently, significant advances have been made in extrapolating toxicity data across species to better understand the potential risks of chemicals to a variety of organisms. However, many of these extrapolation methods do not take into account the evolutionary relationships among species. Since shared evolutionary history is often highly associated with similary physiology, it is generally expected that sensitivity to chemicals should be similar among closely related species. We examine the phylogenetic basis of sensitivity to a variety of chemicals across fish using existing data on acute toxicity (i.e., LC50). Preliminary analyses suggest that the degree of phylogenetic signal in toxicity is highly variable across chemicals and may be a result of both real biological factors (e.g., chemical mode of action) as well as methodology (e.g., sample size).

414 A Petri Net Model for Physiologically Based ToxicoKinetics (PBTK) of Waterborne Fluoranthene in Rainbow Trout

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Physiologically Based Toxicokinetic (PBTK) Modeling has been instrumental in describing the Absorption, Distribution, Metabolism, and Excretion (ADME) of anthropogenic compounds in indicator species for decades. The models rely on relatively simple, straightforward formulas of mass balance uptake, blood/tissue and blood/water partitioning, metabolic kinetics, etc. As a whole, however, these models become complicated when all the formulas come together in large Ordinary Differential Equations (ODEs) which require programming and mathematical skill in order to solve. This study uses Petri Net (a graphical modeling technique) in Snoopy (an Integrated Development Environment [IDE]) to build and simulate a PBTK model of waterborne Fluoranthene uptake in Rainbow Trout. An existing ODE model was simulated in Matlab with code provided in the literature. A Petri Net model was created in Snoopy and the results compared to the ODE model. The goal is to make PBTK models more user friendly so that investigators can concentrate on the compounds, kinetics, and organisms rather than the programming. Petri Nets also offer advantages in simulation, documentation, and distribution of the models to the scientific community once they are created. In addition, Petri Nets are used extensively in modeling biochemical networks, which could be integrated with the Petri Net PBTK models.

415 Bioelectrical impedance analysis as a predictor of fish health using rainbow trout (Oncorhynchus mykiss)

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Bioelectrical impedance analysis (BIA) was used to create predictive models which could non-lethally estimate the body proximate composition of rainbow trout (Oncorhynchus mykiss). BIA readings were obtained from a mixed sex population of fish consisting of different sizes from which several predictive models were produced. Models predicting dry mass, water mass and total energy content of the fish were created with r^2 values of: 0.9064, 0.9170 and 0.9149 respectively. The effect of electrode position was also tested to determine if the position of the electrodes had an effect on the BIA measurements. Readings were taken along the dorsal, lateral and ventral sides of the trout. Models produced from the dorsal measurements were stronger predictors of body composition than models produced from lateral or ventral measurements. The ability of BIA to determine the sex of rainbow trout was also tested to determine if male and female fish could be separated based on resistance readings from the instrument. No significant difference was observed in the resistance values between male and female fish. Overall, BIA was shown to be an accurate biomarker of rainbow trout proximate composition and would be a useful tool for assessing contaminant effects on fish bioenergetics in both the laboratory and the field.

Experimental and Modeling Approaches to Account for Real-World Complexity in Environmental Toxicology

416 AQUATOX: A quantitative platform to identify and evaluate multiple interacting stressors in complex environments in an intensely farmed watershed

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Agricultural runoff can impact receiving waters in many ways, generally leading to degradation of water quality and adverse effects on algae, invertebrates, and fish, and sometimes on humans through contamination of the food chain. A complex agricultural watershed has many potential stressors of differing impact and amenable to differing levels of mitigation; furthermore, these stressors often exhibit interactions that should be understood if management is to be effective. The AQUATOX model couples aquatic fate, toxicology, and ecosystem submodels to "close the loop," representing both direct and indirect effects (by interrupting the trophic structure of the food web); it also provides a platform to which other environmental stressors, such as sediments, scour, and elevated temperatures may be added for combined analysis. Zollner Creek, Oregon, was chosen as a test case because the watershed is 91% agricultural, with 43 different agricultural land uses identified, including row crops, orchards and vineyards, grain and grass fields, and large poultry farms. In 2006 water quality was found to be impaired for resident fish and shellfish, salmonid fish spawning, and anadromous fish passage. The ODEQ began monitoring pesticides in the creek in 2005; they found a variety of pesticides with a high frequency of detection and some, including chlorpyrifos, exceeded water quality standards. The frequency of sampling was sufficient so the empirical chlorpyrifos data were imported directly from the USGS file; the only conditioning of the data was to substitute very low concentrations when data were below detection limits. Total suspended solids were not identified in the TMDLs as an impairment, but they are a factor in nutrient and pesticide transport and bioavailability. Suspended detritus can cause anoxic conditions with serious impacts on coldwater and anadromous fisheries; both suspended inorganic and organic particles are thus important for an integrated environmental analysis. Because they were not measured continuously, TSS was regressed on discharge, which is measured continuously. Suspended organic detritus values are abnormally high in Zollner Creek; extensive poultry houses and waste disposal on fields can account for most of the detrital contamination. Nitrate concentrations are also high due to agricultural runoff, especially when carrying poultry wastes. We will continue to explore stressor interactions through factorial applications.

417 Assessing the Combined Effects of Metal Contamination and Sediment Deposition on Benthic Invertebrate Colonization

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The combined effects of sediment deposition and metal contamination can pose serious risks to aquatic ecosystems, particularly in mountainous regions. To improve bioassessments and recovery potential of aquatic ecosystems, novel experimental approaches are needed to address the impacts of multi-stressors on biotic indicators. This research examines the effects of metal contamination and sediment infiltration on benthic invertebrate community colonization at the North Fork of Clear Creek, a USEPA Superfund site in Black Hawk, Colorado, USA. Metal-contaminated coarse and fine sediment was transported to an upstream reference site where the rate of colonization by benthic macroinvertebrates was observed for 30 days. In addition to the presence or absence of metal-contaminated sediment, results were interpreted using grain size, organic matter, and species traits. Abundance increased over time for all insect orders; however, these changes were much slower in treatments with metal-contaminated fine sediment. Further analysis from mesocosm experiments showed how patches of metal-contaminated sediment influence downstream community composition. This study indicates a need to focus on interpreting other abiotic variables and species traits when estimating stream recovery. In particular, colonization rate of dominant taxa should also be quantified when assessing ecosystem responses to stream restoration. This research has broad implications pertaining to management strategies and our understanding of benthic community recovery in impaired environments with the use of field experiments.

418 Utilizing Higher Tier Aquatic Exposure Modeling and Monitoring for Pesticide Risk Assessments

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The pesticide industry is highly regulated by the United States Environmental Protection Agency (USEPA) and additionally by some state regulatory agencies. There are significant research demands necessary for the initial registration of a pesticide, as well in maintaining these registrations. Initial environmental fate laboratory data are used in conservative regulatory models to determine the potential exposure in the environment, and these exposures are used to determine potential risk to ecological systems. If predicted exposure exceeds the level of concern for a specific organism (e.g. non-target plants, aquatic organisms, etc.), higher-tier modeling can provide a more refined, 'real-world' exposure, however, there are regulatory limitations on the use of these refinements in the regulatory risk assessments. To resolve the uncertainty of the risks based of these modeled exposure estimates, higher-tier real-world studies and monitoring are necessary. Examples of the conservative nature of the regulatory models in aquatic assessments will be compared to real-world monitoring data -- including data generated by non-industry sources such as federal and state monitoring programs. The value and limitations of the modeling and monitoring data in the context of a real-world risk will be evaluated.

419 Building a Predictive Adverse Outcome Pathway for Multiple Acetylcholinesterase Inhibitors to Predict Effects to Population Scale Endpoints

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One of the major issues in environmental toxicology and risk assessment is the integration of molecular interactions to effects observed at landscape scales. Adverse outcome pathways (AOPs) are linear acyclic models to describe the interaction of a toxicant with the molecular, cellular, organs and eventually the organism. Currently AOPs do not predict population scale effects with the context of the landscape. This presentation describes how to extend the AOP framework to a Bayesian network to describe interactions at each biological scale to derive the key parameters for estimating population dynamics. These parameters are survival and timing to first reproduction, survivorship between age classes, and reproduction at each age class. Bayesian networks will be parameterized to include molecular interactions including synergistic and antagonistic effects. Age structured population models are used to incorporate ecological context to include carrying capacity, density dependent or independent population control, and patch dynamics. The initial examples are different organophosphate pesticides both individually and in mixture. The modes of action are clear; synergism has been identified and has well defined exposure-response curves. Finally we will present how this approach can be extrapolated to the estimates of effects and risk to endangered species, salmon in the Salish Sea.

420 Mixtures of Chemical Pollutants at Environmental Concentrations: How to deal with?

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Thousands different chemicals are discharged into the environment from agriculture, industry, medical facilities, house-holds. Currently, there is an increasing concern for the environmental impact of mixture of compounds since the additive and eventual synergistic effects are unknown and could produce serious adverse effects. Recently, the European Commission has issued a Communication on combination effects of chemicals highlighting the need to ensure that risks associated with chemical mixtures are properly understood and assessed. To address this issue, a joint-effort of 16 European and associated research groups participated to an exercise to test a synthetic reference chemical mixture on the own routine bioassays to investigate the chemical mixtures effects (Carvalho et al., 2014)). The reference material was prepared by ISPRA (Italian reference Laboratory for chemical) included class of pesticides, pharmaceuticals, industrial products, heavy metals and polyaromatic hydrocarbons such as atrazine, diuron, isoproturon and simazine (herbicides), benzo[a]pyrene and fluoranthene (polycyclic aromatic hydrocarbons), cadmium and nickel (metals), DEHP (plasticizer), 17β-estradiol (naturally occurring estrogen), 4-Nonylphenol (surfactant), diclofenac (pain killer), chlorphenvinphos and chlorpyrifos (insecticides), and the emerging compounds bisphenol A, carbamazepine, sulfamethoxazole, triclosan and DEET. The mixures were prepared, each compound at Equivalent Quality Standard (EQS) value, the safety limit concentration allowed by the European Water Framework Directive (WFD). The bioassays covered the entire ecosystem from bacteria to fish as well in vitro assays providing an unique scenario from ecological risk assessment perspective. The results showed that effects were observed at very low concentration on algal-bacteria composition in a marine microcosm, immobilization in crustacean, fish embryo toxicity and frog embryo development. Transcriptomics was performed for the marine diatom Thalassosiria pseudonana exposed either to single compound or the mixture showing that the effects of the mixtures could be explained mainly by the combined exposure to the four herbicides. Preliminary results will be presented. We conclude that some precaution on the chemical mixture assessment should be taken even in case the individual compounds are present at EQS, the safety limit concentration under European legislation.

421 Restoration Scaling of Lost Ecosystem Services in Complex Aquatic Systems

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Environmental regulations in the U.S. and Europe require compensation of lost environmental services due to releases of hazardous substances,

oil spills, and other perturbations. Habitat Equivalency Analysis (HEA) has often been used to scale present value of lost services with compensatory restoration. HEA assumes that all areas within a habitat category are functionally equivalent and exchangeable. This can create challenges for complex aquatic sites for addressing issues associated with the biology and life history of the species, the dynamics of habitat, life history and functional connectivity, complex hydrology, and non-stationarity. HEA can be adjusted to align with the complexity of the environmental conditions to support settlements. However, difficulties in aligning HEA with complex environmental conditions can create obstacles to scaling services loss with compensatory restoration and impair settlement negotiations. For example, responsible parties can find it difficult to get management approval of a large settlement when the basis of the settlement is a simplified analysis of complex conditions. The objective of this presentation is to present an alternative approach for restoration scaling of lost services in complex aquatic systems. We suggest a habitat-based approach aligned with the life cycle of indicator species for evaluating complex aquatic systems. Such an approach allows consideration of habitat change in the context of the species life history and the interactions that occur across biological and physical scales. For example, the Ecosystem Diagnosis and Treatment model was developed to support species recovery and restoration planning for various salmonid species and has been used extensively for restoration planning. The model has been used to evaluate both the preservation and restoration potential associated with the impacts of various chemical, physical, and hydrological perturbations. The software code is publically available and editable making it possible to align the analysis with site-specific issues. Importantly, the model evaluates habitat change in biologically meaningful terms such as the change in potential productivity, capacity, abundance and life history diversity of the population as a function of habitat conditions. In addition to use for restoration scaling, the model provides an transparent framework for selecting and quantifying potential restoration alternatives, including geospatial implications.

422 Asymmetric effects of pharmaceutical exposure genarate unexpected ecological effects

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Pharmaceuticals designed to modify behavior are found in waterways globally, but how these affect aquatic organisms and food webs is largely unknown. We have priorly shown that pharmaceuticals may invoke asymmetric effects when comparing species on different trophic levels, such as invertebrates and fish. Here we present results showing for the first time that pharmaceutically induced behavioral modifications differ between wild fish species, resulting in species-specific increases in predation risk, with ecosystem-level impacts. We found that, activity and boldness increased significantly in Eurasian perch (Perca fluviatilis), resulting significantly higher predation risk, while behaviors and predation risk of crucian carp (Carassius carassius) were unaffected. However, counter to our expectations, a tri-trophic level model based on our results show adverse impacts on the unaffected crucial carp, the top predator, and depletion of the basal resources. As such, our results indicate that asymmetric effects of pharmaceutical contamination may cascade both up and down aquatic food webs, with implications for overall ecosystem functioning.

423 Comparative potency and mechanisms of four model industrial chemicals to induce antioxidant and oxidative stress gene expression in zebrafish larvae

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The question of what chemicals cause oxidative stress, and through which molecular pathways the oxidative stress generated by those chemicals acts, is an important one for toxicologists. In order to investigate the importance of the Nrf2 pathway in sensing and responding to oxidative stress in zebrafish, we examined changes in larval gene expression induced by exposure to four chemicals: two different peroxides (tertbutyl hydroperoxide and cumene hydroperoxide); and two potential Michael acceptors (hydroquinone and R-(-)-carvone). For each chemical, we analyzed expression of an array of ten antioxidant/oxidative response genes and three reference genes using the Affymetrix QuantiGene Plex (QGP) platform. The zebrafish genes contained in our array included those considered Nrf2 (Nfe2l2a)-dependent (hmox1, gstp1, gclc); potentially Nrf2-dependent (nqo1, prdx1, gpx1a); genes modulated by oxidative stress (sod1, sod2); genes responsive to cellular stress and DNA damage (hsp70, gadd45bb), and 3 reference genes (actb1, gapdh, hprt1). We measured expression of each gene in response to a range of concentrations of each chemical based on the LC_{50} of that particular chemical in embryonic/larval zebrafish. We used these data to compare the modes of action of the two pairs of chemicals. For the hydroperoxides, we modeled the O-O bond strength and radical stability. For hydroquinone and R-(-)-carvone, we modeled the relative reactivity and compared to the patterns of gene expression change caused by each to determine whether they both undergo Michael addition as the first step to causing oxidative stress, or whether hydroquinone instead causes oxidative stress through a radical mechanism. We then compared changes in gene expression of wild-type fish to those of fish harboring a mutation in the DNA binding domain of nfe2l2a (the mutant line nrf2^{fh318}), and used these data to predict the extent to which each chemical activates the Nrf2 response pathway. Our results demonstrate the power of combining genetic analysis and modeling to understand the complexities of chemical effects on whole organisms. Supported by NSF 339637 and NIH P42-ES04696.

Advanced Analytical Methods for Contaminant Discovery

424 Exploring environmental chemical space through HR/AM mass spectrometry and cheminformatics: The example of wastewater-derived organic micropollutants

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High-resolution accurate-mass mass spectrometry (HR/AM MS) provides a promising approach for comprehensive structural elucidation of numerous trace-level organic contaminants in aquatic media. However, comprehensive sample characterization is made difficult by inefficiencies in the prioritization of detected features and postulated structures based on tandem MS analysis. We have developed a holistic, non-targeted screening workflow to address these challenges, which combines ultra-high resolution tandem mass spectrometry, computational mass spectrometry. We illustrate the utility of this approach to evaluate the occurrence of organic micropollutants in the effluent of a conventional domestic wastewater treatment facility. Composite effluent samples from the North Durham Wastewater Reclamation Facility (Durham, NC, USA) were analyzed using an Orbitrap Fusion tribrid tandem HR/AM mass spectrometer. Resulting data were aligned and componentized to yield 3,701 unique features, for which >91% had associated tandem mass spectral data. Custom data processing scripts were deployed to pipe relevant feature data to computational mass spectrometry tools for molecular formula assignment based on isotope pattern and fragment spectrum decomposition (SIRIUS, formulas assigned to 90.2% of features), structure assignment from the PubChem compound repository (postulated structures returned for >77% of features) and automated tandem mass spectral annotation and scoring (MetFrag CL, MAGMa, and CFM-ID). Finally, postulated structures were scored based on their structural similarity to possible environmental contaminants (i.e., compounds known to be produced in commerce) and to known wastewater pollutants. Structure descriptors were calculated using 42-molecular quantum numbers (MQN), a unique descriptor set that implements simple atom, bond and topology counts. After rigorous data filtering, 607 micropollutants were tentatively identified, representing classes of pharmaceuticals, transformation products, consumer chemicals, and industrial compounds. Overall, our results highlight the benefit of combining of state-of-the-art computational mass spectrometry tools with chemoinformatic approaches for increased efficiency and annotation rate in non-targeted analysis of built and natural environments.

425 Nontarget Analysis of Polar Organic Chemical Integrating Sampler Extracts--complementary tools for unknowns analysis

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Comprehensive identification of known and suspected organic contaminants in surface water systems relies on sampling designs that reliably collect the fullest range of contaminants present. Integrating passive samplers, such as the polar organic compound integrative sampler (POCIS), provide the means to collect an integrated profile of many polar organic contaminants present in surface water whose presence may be continuous or episodic, reflecting changes in hydrologic or source input. Coupling POCIS with a comprehensive nontarget analysis scheme provides both qualitative identification and semiquantitative concentration estimation of targeted and nontargeted contaminants and permits comparing POCIS-derived contaminant profiles to assess varying presence of argeted and nontargeted contaminants. We present results from the structured nontarget analysis of POCIS extracts initially analysed using a targeted method for the determination of 109 pharmaceuticals. The extracts were analysed using a Waters Synapt G2S Quadrupole Time-of-Flight Mass Spectrometer (QToF MS) operated in the positive electrospray ionization mode and coupled to a Waters Aquity Ultra Performance Liquid Chromatograph, using reversed phase separation for separation of analytes. The QToF MS was operated in a scan mode combining spectra with alternating low and high collision cell energies. The resulting data were then aligned and analysed using Waters software and an extended pesticide and toxicology library to identify known and suspected contaminants. Results from the QToF analysis were compared to targeted analysis using high-performance liquid chromatography/tandem quadrupole mass spectrometry. Methyl-1H-benzotriazole, carbamazepine, citalopram, desvenlafaxine, fexofenadine, and tramadol were among the compounds in POCIS extracts detected by LC/MS/MS and confirmed by QToF MS. Lamotrigrine and DEET, not determined by the LC/MS/MS, but anticipated to likely be present, were identified by QtoF MS from elemental compositions of the parent pseudomolecular ions and the associated fragments from the linked high-energy collisional dissociation spectra. The hydroxyl degradation products of carbamazepine and lamotrigrine were similarly identified in these extracts. Procedures to identify these and other organic contaminants and contaminant degradates in POCIS extracts are further discussed, providing a clear demonstration of the value of combining POCIS and QToF MS for non-target analysis.

426 Occurrence of suspect and non-target contaminants and their transformation products in a linked surface water system influenced by treated wastewater

A. Huba, Florida International Univ / Chemistry Biochemistry; P.R. Gardinali, Florida International Univ / Chemistry Biochemistry and SERC

Due to the finite nature of the world's fresh water sources, the recycling of treated wastewaters has been a significant area of development in the past years. Water recycling, however, leads to considerable apprehension with respect to the presence and persistence of unregulated contaminants that are introduced through treated wastewater releases. Water treatment plants, although designed to remove a variety of contaminants, are not always efficient and may, additionally, introduce new products which are formed during the treatment. The monitoring of the composition of the incoming and outgoing water is therefore always important. Owing to the recent development of increasingly sensitive and selective analytical tools, non-target screening methods have gained significant interest. These approaches allow for a more comprehensive screening since the a priori selection of compounds is no longer needed, opening the possibility for retrospective analysis. The potential of suspect and non-target screening workflows was here demonstrated for the analysis of contaminants in surface and drinking water in a typical system impacted by a treated domestic effluent. High performance liquid chromatography-high resolution mass spectrometric (HPLC-HRMS) analysis was carried out on an Orbitrap Q Exactive, and subsequent data processing included peak picking, retention time alignment, blank subtraction, elemental formula assignment, ChemSpider search and MS/MS spectral matching, with the use of the Compound Discoverer 2.0 software, as well as the MassBank library, and the in silico fragmenter MetFrag. Suspect and non-target screening was able to tentatively identify over 70 compounds, including pharmaceuticals, plasticizers and other domestic use based contaminant classes, many of which were shown to be persistent along the entire system. Analysis of the drinking water treatment plant influent and effluent showed the presence of 242 not efficiently removed components (including the tentatively identified DEET, 4-nonylphenol, and hexamethoxymethylmelamine), as well as 269 newly formed components, likely by-products of the treatment process. Further analysis also yielded the identification of transformation products, including cotinine and O-desmethylvenlafaxine. Moreover, Kendrick mass defect plots were used to identify potential areas of interest, such as heteroatom containing byproducts, which represent an area for further development of advanced treatment technologies.

427 Evaluation of Emerging Contaminants in Great Lakes Fish using comprehensive two-dimensional gas chromatography high resolution mass spectrometry

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The Great Lakes Fish Monitoring and Surveillance Program (GLFMSP) has traced the fate and transport of anthropogenic chemicals in the Great Lakes for decades. The contemporary impact of emerging contaminants was recently recognized and the program has dedicated significant resources to understanding the threat posed by non-legacy toxic chemicals. The identification of novel contaminants in top predator fish is a major challenge due to the complex biological matrix. Traditional legacy chemical purification steps can potentially discriminate against unknown toxic chemicals present in the original extracts. To enhance the comprehensive screening ability of GLFMSP, top predator fish extracts have been analyzed using a state-of-the-art two-dimensional gas chromatograph coupled to a High Resolution Time of flight mass spectrometer (GCxGC-HRMS, LECO GC-HRT). Due to the increased peak capacity and mass accuracy offered by GCxGC, and HRT, respectively, fish tissue extracts were injected with minimal sample cleanup. Identifications were performed by library spectral matching and molecular formula estimations using high resolution data. The efficacy of this technique as a targeted screening/confirmation and non-targeted discovery tool will be assessed with a summary of novel chemicals observed in Great Lakes top predator fish.

428 Chemical characterization of indoor dust by comprehensive target and non-target screening using GC- and LC-QTOF-MS/MS

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House dust is contaminated with a broad range of chemicals such as pesticides, personal care products, plasticizers, flame retardants, and polyfluorinated compounds. It can serve as a marker of exposure to humans and is known to be a reservoir for many released compounds. Previous studies have focused on investigating one or several compound classes in a targeted analytical approach. The recent development of high-resolution mass spectrometers and corresponding software offers the possibility of screening for suspected or non-target chemicals. The goal of this study is to comprehensively characterize the chemical fingerprint in 50 dust samples collected from two groups of households: families with normal developing children and families with children having developmental issues. Over 130 frequently used chemicals that span the chemical space were selected to optimize the sample preparation and analytical methods. The final method consisted of sonication extraction using hexane and acetone without further sample clean-up. One part of the extract was measured on an Agilent GC-QTOF-MS using electron impact (EI), while the other extract was analyzed on an Agilent LC-QTOF-MS using electrospray ionization (ESI) in positive and negative mode. Absolute recoveries were above 50% for more than 95% of the chemicals, and method detection limits were below 100 ng/g for 80% of the chemicals. Following the quantification of the target chemicals, the dust samples were screened for several thousand additional compounds of interest in dust using different databases. Thereby, exact mass screening and MS/MS spectra comparison for chemicals measured by LC-QTOF-MS as well as comparison with library spectra for chemicals measured by GC-EI-QTOF-MS was used for the detection of these suspected chemicals. Finally, all features in the samples were extracted in a recursive way. Mass Profiler Professional (MPP, Agilent Tech.) was used to prioritize non-target features of interest, i.e. features with highest detection frequencies and features with significantly different abundances in samples from the two different household groups. Prioritized features were tentatively identified by MS/MS measurements, the use of the MetFrag software tool and the DSS-TOX database (EPA) containing over 100,000 household related chemicals. This multi-step screening will give new insights in the chemical fingerprint of US indoor dust by identifying unknown chemicals that should be further investigated.

429 Streamlining Non-Targeted GCxGC/TOFMS Data Analysis of Remediated Soil Samples using Python

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Bioremediation of polycyclic aromatic hydrocarbon (PAH) contaminated soil samples may lead to the formation of polar toxic PAH transformation products, many that are non-routinely monitored. To identify these transformation products, an effect-directed analysis approach was used to determine toxic fractions of bioremediated samples. These toxic fractions were analyzed using comprehensive two-dimensional gas chromatography coupled to time-of-flight mass spectrometer (GC×GC/TOFMS) and ChromaTOF® software from LECO. While this non-targeted analysis approach is suitable for elucidating unknown compounds that cause increased toxicity following bioremediation, the list of potential compound candidates were in the range of tens of thousands. A Python program was developed to automate and accelerate the identification of the compounds in these toxic soil fractions. The program takes exported data from LECO ChromaTOF®'s Statistical Compare feature, which is a peak alignment tool that also show peak variations between groups, and returns a list of potential compounds with increased peak responses post-bioremediation. The peak tailing factor and the compound peak response before and after bioremediation were used as the parameters to identify candidate compounds. Comparison between the results from the script and manual identification were made. One of the fractions was the most toxic and comprised of over 1,000 compounds. The Python program select candidate compounds, based on peaks identified by Statistical Compare, within minutes, while manual selection required several days of pruning. In addition, the Python program standardizes the analysis of complex PAH mixtures in the environment by reducing the potential for human variability. Moreover, this program can be adopted across multiple laboratories and disciplines. Altogether, a Python script was written that streamlines the identification of compounds that are formed following bioremediation of soil samples by making the process more time efficient. While the program was written to determine PAH transformation products that were formed following bioremediation, the script can be modified to identify PAHs that were degraded as well. This program can aid researchers that use GC×GC-TOFMS for non-targeted analysis of chemicals in the environment by reducing the analysis time.

430 Identification of Novel Brominated Disinfection By-Products of Concern by Use of DIPIC-Frag Untargeted Screening and Effect-Directed Analysis

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Disinfection byproducts (DBPs) are produced during drinking water treatment processes as a result of the reaction of disinfectants with natural organic matter (NOM). Brominated DBPs (Br-DBPs) are a specific class of concern due to consistent reports that they are more toxic than their chlorinated analogues; however, a large proportion of Br-DBPs in drinking water remained unidentified and subsequently uncharacterized. Identification of Br-DBPs in actual drinking water samples is challenging due to complicated interferences, the extremely low concentrations at which Br-DBPs exist, and their chemical diversity. To overcome these challenges, we adopted a data independent precursor isolation and characteristic fragment (DIPIC-Frag) method for the untargeted screening of Br-DBPs in drinking water. Analytical conditions were compared including sample pretreatment methods (SPE and freeze-drying), ionization modes (APCI and ESI), and HPLC columns (C18 and Amide) to expand the coverage of Br-DBPs detected. 1,857 Br-DBPs peaks were robustly detected by the DIPIC-Frag method and analytical conditions were deemed complimentary. Precursor ions and compound formulas for 1,303 Br-DBPs were predicted and structures were proposed for the 50 most abundant peaks. This represents the largest mass spectrometry library of Br-DBPs established to date, and most of these Br-DBPs have not been previously reported. To investigate what water treatment processes contribute to the formation or removal of Br-DBPs, water samples collected from each stage of treatment at two different plants located in Saskatchewan were compared. Then, effect-directed analysis was performed by use of the CHO-K1 cell line, with cytotoxicity and DNA damage being the toxic endpoints of interest. The most toxic fractions were determined and mass spectrometry results were used to identify novel Br-DBPs that likely present the greatest concern in drinking water.

431 Progress in the analysis of chlorinated paraffins – different methods and interlaboratory study results

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Chlorinated paraffins (CPs) complex industrial mixtures of >1000 polychlorinated n-alkanes, are globally being produced in high volumes of more than 1 million metric tonnes per year. They have many applications such as flame retardants, and additives in metal cutting fluids. The short chain CPs (SCCPs) have been placed on the persistent organic pollutants (POPs) list of the Stockholm Convention. They need to be monitored in various matrices, but, still, reliable analytical methods are lacking. Some information is available for methods for SCCPs, but conclusive data is very limited for medium-chained CPs (C14-17; MCCPs), and information on long-chained CPs (C>18; LCCPs) is entirely lacking. The suitability of the current instrumental techniques to determine these compounds in environmental samples and the potential to improve this determination needs to be investigated. We evaluated various instrumental techniques for the determination of SCCPs, MCCPs and LCCPs, for ease of use, analysis time, sensitivity, selectivity, repeatability, reproducibility, and approximation of the true CP concentration. To achieve this, a range of the most promising and currently most applied instrumental techniques were selected (e.g. GCxGC-ECD, GC-ECNI/LRMS, GC-EI/QQQMS, qTOF-MS). CP mixtures and environmental samples were analysed and methods validated. In addition, we organized three rounds of an international interlaboratory study and results of those studies, in which ca. 20 laboratories participated, will be discussed.

Climate Change and Water Resource Management: An Ever-Changing Challenge

432 Life in a Warmer More Acidic World: Physiological Implications of Climate Change on Aquatic Organisms

K. Brix, EcoTox / Zoology

Global climate change presents the single greatest environmental challenge of the 21st century. The Intergovernmental Panel on Climate Change (IPCC) has issued several reports making projections of future climate scenarios, largely dependent on the magnitude of CO_2 emissions for the remainder of this century. While there are uncertainties in these projections, it is clear that the climate has changed, and will continue to change, at a rate that has only been observed a few times over the last 300+ million years. Although the magnitude is uncertain, in aquatic systems we can expect elevated atmospheric CO_2 to result in warmer (~2-4 °C increase) more acidic (~0.2-0.4 pH decrease) waters. An overview of these projected changes in temperature and pH in aquatic systems is presented. Increases in temperature and acidity can directly affect multiple physiological processes including metabolism, oxygen transport, acid-base balance, ion transport, and oxidative stress. An overview of these effects will be presented with the objective of providing a physiological framework of how climate change may affect organisms from which to evaluate the interactions between climate change and other anthropogenic stressors.

433 Assessing Climate Change Effects on Freshwater Fish Distribution Using a Habitat Suitability Model

T. Shim, J. Jung, Korea Univ / Environmental Science and Ecological Engineering

Water quality and quantity are affected by climate change due to temperature and precipitation changes, which alters the habitat suitability of aquatic organisms. In order to predict effects of climate change on freshwater fish distribution, an ecological habitat suitability model (EHSM) was developed in this study. The EHSM is based on the concept of a habitat-hydraulic model, physical habitat simulation system (PHABSIM), with accommodation of a habitat-physiologic model, CLIMEX. The EHSM utilizes hydraulic variables (velocity, depth, substrate) and water quality variables including temperature to assess the habitat suitability of freshwater fish. Zacco platypus was selected as a model species because they are commonly found in Korea. Overall, the habitat suitability of Z. platypus tends to increase with increasing temperature by climate change, mainly due to their broad thermal habitat preference.

434 Protecting Aquatic Life from Effects of Streamflow Alteration

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Protecting aquatic life from the effects of streamflow alteration involves maintaining multiple components of the flow regime within their typical range of variation. The U.S. Environmental Protection Agency (EPA) in collaboration with the U.S. Geological Survey have developed a report to serve as a source of information for states, tribes, and territories on: 1) the natural flow regime and potential impacts of flow alteration on aquatic life; 2) Clean Water Act programs that can be used to support the natural flow regime and maintain healthy aquatic ecosystems; and 3) a flexible, non-prescriptive framework to quantify targets of the flow regime that are protective of aquatic life. This flexible framework incorporates EPA guidelines for ecological risk assessment and relevant concepts from contemporary environmental flow science literature. The framework does not prescribe any particular analytical approach (e.g., statistical modeling), but rather, it focuses on the process, information, and data needed to evaluate relations between flow and aquatic life and to select appropriate numeric flow targets. While this paper deals primarily with streams and rivers, non-flowing systems (e.g., lakes and wetlands) are inextricably connected to flowing systems and can therefore benefit from measures to maintain hydrologic conditions in flowing water bodies.

435 Ecotoxicological impact of re-mobilized sediments and flood events for look regulated rivers and wetlands

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In recent years, sediments have become a central topic of scientific and public discussion as an important factor for determining water quality. While the quality of surface waters in Germany has significantly improved during the past years, highly contaminated sediments still create a considerable threat to the quality of several European catchment areas. For several European river basis, including Neckar, Rhine and Elbe, highly contaminated old sediments can be described as “potential chemical time bombs”. An important process which may remobilize such sediments and which is still of increasing importance in relationship to the global climate change is more often occurrence of stronger floods in Europe as well as in other parts of the world. To understand and predict possible toxicological and ecotoxicological consequences of contaminants mobilized from sediments by flood events it is necessary to develop scientific approaches for the assessment of regularly flooded rivers. The combination of hydrodynamics and ecotoxicological investigations is devolving to an emerging field of research. Recently, it was shown that hydrodynamic aspects can be involved as additional line-of-evidence in Weight-of-evidence studies assessing the impact of sediments. In the last decade several studies were published addressing the ecotoxicological impact of flood events or using combined approaches for evaluating flood events and the risk of erosion. Here, we summarize different concepts and case studies for the assessment of sediment quality and report on some novel integrative test methods for assessing sediment toxicity including contaminant re-mobilization during simulated re-suspension events. Within the talk several case studies addressing the ecotoxicological impact of re-mobilized sediments and flood events for look regulated rivers and wetlands are presented. Combined investigations of sediment contamination and mobility as well as acute and mechanism specific biotests in effect directed analyses/weight-of-evidence studies show great potential for the assessment of chemically polluted rivers and should be included into the ‘programmes of measures’ within future management concepts.

436 Indirect effects of climate change on Zinc cycling in sediments: the role of changing water levels

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Increased variability in lake and river water levels associated with changing climate, could impact the fate and effects of metals in redox sensitive sediments. Sediment metal binding is affected by a fluctuating water table through the alteration of microbial communities, acid-base and redox chemistry. The objective of this study is to determine the influence of water level fluctuation on metal speciation in pore water and predict environmental risk to benthic communities in high carbonate systems. We collected sediments from four metal contaminated coastal freshwater wetlands in Michigan and constructed experimental wetland microcosms. Water level fluctuation experiments emulated a short-term (seiche) and 32-day drought scenario. Porewater and sediment metals (Ca, Cu, Fe, Mg, Mn, Ni, Zn) and important metal binding phases (iron-oxide speciation, acid-volatile sulfide) were quantified. During the 32-day drought experiment, upon re-inundation of oxidized sediments, an increase in porewater Cu, Zn, Mg, Ca, was observed for most sites, after which the concentrations decreased slowly over 16 days. In lab studies porewater Zn increased upon inundation to levels exceeding the USEPA threshold for chronic and acute toxicity (mean = 219.2 ± 19.8 µg L^{-1}). These data show the dissolution of iron oxide bound Zn and kinetically slow reformation of metal sulfides contributes to the bioavailable metals after re-flooding. This study suggests increased ecological risk from metals to organisms present in drought sensitive regions. Climate induced changes in regional hydroperiods are likely to alter the bioavailability of metals to aquatic organisms.

437 The deal with diel: Effects of temperature fluctuations, asymmetrical warming, and ubiquitous metals contaminants on three amphibian species

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Climate projections over the next century include disproportionately warmer nighttime temperatures ("asymmetrical warming"). Cool nighttime temperatures lower metabolic rates of aquatic ectotherms. In contaminated waters, areas with cool nights may provide thermal refugia from high rates of daytime contaminant uptake. We exposed Cope's gray tree frogs (Hyla chrysoscelis), southern leopard frogs (Lithobates sphenocephalus), and spotted salamanders (Ambystoma maculatum) to five concentrations of a mixture of cadmium, copper, and lead in filtered lake water under three to four temperature regimes, representing asymmetrical warming. At concentrations with intermediate toxicosis at test termination (96 h), temperature effects on acute toxicity or escape distance were evident in all study species. Asymmetrical warming (day:night, 22:20 ^{o}C; 22:22 ^{o}C) doubled or tripled mortality relative to overall cooler temperatures (20:20 ^{o}C) or cool nights (22:18 ^{o}C). Escape distances in treatments with the same water quality were 40 to 70% shorter under asymmetrical warming. Results suggest potentially grave ecological impacts from unexpected toxicosis under climate change.

438 Exploring the impacts of multiple anthropogenic and environmental stressors: data needs for predicting ecological effects

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The Organization for Economic Co-operation and Development (OECD) provides standardized guidelines for testing pesticide toxicity. For aquatic invertebrates, the cladoceran Daphnia magna is used as a model organism and is typically exposed to a range of chemical concentrations at a constant and optimal temperature for 48 hours (acute) or 21 days (chronic). The guidelines for these tests simplify experimental design and allow for straightforward data analysis to inform environmental regulations. Although these tests hold value, they do not consider fluctuations and variations in other environmental factors that are commonly experienced in nature. For example, organisms exposed to pesticides in natural water bodies are likely to experience non-optimal temperatures, variable food conditions and intra-specific competition along with a plethora of other stressors. In this study, D. magna was used to test the toxicity of the fungicide pyraclostrobin under different temperature regimes and at different conspecific densities. Pyraclostrobin is a strobilurin fungicide used nationally to combat 19 fungal diseases that impact crop yields. The objectives were to: (1) determine the toxicity of D. magna to pyraclostrobin under acute and chronic exposure conditions, (2) determine if elevated temperatures alter pyraclostrobin toxicity, (3) determine if conspecific density and competition for food alter toxicity to pyraclostrobin and, finally, (4) evaluate how results from standard toxicity tests compare to results from multi-stressor conditions. So far, our results show that pyraclostrobin toxicity increases with temperature (> TEMP) and that daphnids are very sensitive to conspecific density with lower survival and reproduction at 4X the control density. These results will inform the study design to evaluate the impacts of all three stressors simultaneously. We will also evaluate the utility of different multi-stressor models to determine if, by combining single stressor test results, we obtain insight into simultaneous exposure effects. Our overarching hypothesis is that standard toxicity assays only provide limited insight into effects that can occur in natural populations that also experience other, disparate stressors.

439 Acute thermal challenge potentiates benzo-a-pyrene-induced cardiac and metabolic toxicity in juvenile rainbow trout (Oncorhynchus mykiss)

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Fish are facing increasing impacts not only from aquatic contaminants, but simultaneously also from thermal stresses due to global warming. Increasing our understanding of their interaction is critical to predict future impacts on fish populations. Acute thermal challenges are known to increase metabolic rate that in turn increases heart rate, cardiac output and fatty acid oxidation except at high temperatures where fish switch to anaerobic glycolysis as cardiac output fails. In contrast, the petrogenic polycyclic aromatic hydrocarbon, benzo-a-pyrene (BaP) causes cardiorespiratory toxicity by also increasing oxygen consumption, but leads to cardiac arrhythmias (atrioventricular blockade), decreased ventricular contractile rate and impaired cardiac output. We hypothesized that an acute thermal challenge would exacerbate the cardiometabolic toxicity of acute BaP exposure in juvenile rainbow trout (Oncorhynchus mykiss). Trout were injected with BaP (i.p. on Days 1 and 2 with 0, 0.1 and 1 mg/kg; n=20 fish/group), then examined at Day 4 for cardiac function (high frequency echocardiography) and metabolic toxicity (plasma lactate, glucose, glycerol and triglycerides). Half of the trout were subjected to acute thermal challenge (3°C increase every 30 min from 12-30°C) and half were kept at 12°C for an equivalent time. In response to thermal challenge, control trout increased heart rate and decreased stroke volume, yet were able to maintain cardiac output until >27°C. In contrast, fish exposed to increasing BaP concentrations showed an impaired stroke volume at all temperatures and depressed heart rate plus arrhythmias >21°C, leading to consistently depressed cardiac output. Plasma lactate was decreased by thermal challenge, but unaffected by BaP. Glucose decreased with increasing BaP, but only in fish without thermal challenge. Moreover, while plasma triglycerides were unaffected by either thermal challenge or BaP, the ratio of free glycerol to triglycerides increased with increasing BaP dose in only thermally challenged trout. In conclusion, acute thermal challenge potentiated the ability of BaP to induce arrhythmias, depress cardiac output and alter metabolism in juvenile rainbow trout. This illustrates the potential for global warming to enhance sensitivity of trout to acute BaP toxicity and the need to re-evaluate toxicity of contaminants in the context of this environmental stressor.

Microplastics in the Aquatic Environment: Fate and Effects – Part 1

440 A Framework for Dynamic Estimation of Environmental Concentrations of Microplastics in WWTP Effluents and Receiving Waters at a National Scale

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Down-the-drain exposure models provide a valuable screening-level tool for estimating environmental exposure to product ingredients which are treated and discharged at municipal wastewater treatment plants. Microplastics, plastic particles smaller than 5 mm diameter, enter wastewater treatment plants (WWTP) due to a variety of sources. Exposure modeling was performed using the iSTREEM® model, a publicly-available web-based model supported by the American Cleaning Institute (www.istreem.org) which estimates spatially-explicit concentrations of chemicals in effluent and receiving waters across the U.S. WWTP influent loadings of microbeads were estimated using per-capita usage derived from market manufacture survey (Gouin et al 2015) combined with individual facility population served and flow estimates within the iSTREEM® model. The analysis used multiple values for removal during treatment based on total suspended solid removal data and a wide range of in-stream decay rates, resulting in a variety of potential environmental exposure estimates. The removal and decay rates have a non-linear effect across the varying facilities & stream segments in the US landscape. Therefore, we developed an approach which leverages the advantages of the iSTREEM® model (national scope, individual facilities, and distributions of output) with the ability to screen for potential concern based on uncertain (and dynamic) removal and decay rates. This allows for flexibility in modeling the environmental concentration of microbeads regardless of size, weight, or physicochemical properties. We developed a 2-dimensional matrix with removal rate and decay rate as the primary axes. The individual cells within the array will then correspond to a reasonable worst-case Predicted Environmental Concentration (PEC) (e.g. 95th percentile) based on the national iSTREEM® results. These concentrations are based on a proxy quantity of microbeads which can be easily scaled. Therefore, with the matrix it is possible to supply an approximate removal and decay rate for the microplastic of interest and assess the estimated exposure by scaling the matrix value using the relationship between the substance quantity of interest and the proxy. This matrix framework can be used to help inform environmental exposure assessments by readily providing concentrations based on varying model inputs on WWTP removal and in-stream decay rates for microplastics, which continues to evolve as more research is conducted.

441 Sorption of triclosan and methyl-triclosan to microplastic and potential for facilitated release from wastewater treatment plants

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Microplastics can be sourced from synthetic clothing, personal care products such as toothpaste or facial cleansers, or by the breakdown of larger plastic debris in the environment. Wastewater treatment plants serve to collect and concentrate wastes that have been shown to contain microplastics in addition to other contaminants of concern, such as triclosan (TCS), an antimicrobial agent common in personal care products, and its metabolite, methyl-triclosan (MTCS). The objective of this research is to measure the microplastic-water distribution coefficients for TCS and MTCS to polyethylene (PE) and a suite of textile fibers (polyester, nylon, polyester-cotton blend, and cotton) in spiked, aqueous batch tests in order to determine to what extent different microplastics can accumulate contaminants as they travel through wastewater treatment plants before being discharged to surface waters. Distribution coefficients (L/kg) for MTCS to PE and microplastic textiles decreased in the order: PE, nylon, polyester, polyester:cotton (65:35) blend, and cotton. Distribution coefficients for TCS followed the same order except for polyethylene, which was the lowest. Unlike the other materials, polyethylene does not contain any hydrophilic functional groups in the polymer backbone. Triclosan, with its hydroxyl functional group (pKa 8.1), is therefore likely participating in specific sorption interactions with the amide and carbonyl groups in nylon, polyester and cotton as has been observed for dyes. Sorption isotherms will provide further insight into this behavior. The observed distribution coefficients for the polyester:cotton blend could be predicted within 0.4% for TCS and 9.1% for MTCS using a weighted combination of the polyester and cotton values. Knowledge of sorption behavior will be combined with monitoring of microplastic in effluents to estimate the potential microplastic-facilitated release of these chemicals from a wastewater treatment plant.

442 The effects of tributaries on the transport of microplastics in the Hudson River Watershed

I. Krout, Marist College / Environmental Science

A new environmental threat known as microplastics is contaminating the Hudson River Watershed. Microplastics are entering the environment through a number of different means: drainage of personal care products containing microbeads, fragmentation and run off from larger plastic materials and fibers released from washing of nylon or acrylic fabrics. Recent studies have shown a growing presence of microplastics in the Hudson River. Due to their high resistance to degradation the accumulation of microplastics may result in harmfulimpact on human and environmental health. This study investigates the contribution of Hudson River tributaries and their role in transport of microplastics throughout surrounding watershed, directly to the Hudson River. The tributaries in the study were chosen based upon their use, pre-assessed water quality and their location in terms of population density and land usage. The four sampled tributaries were located in the State of New York. Sampling was performed using a flow through technique with a drift net submerged for 15 minutes collecting all particulate matter smaller than 333 µm. In each water sample collected from chosen streams the following water quality parameters were measured; pH, dissolved oxygen, salinity, temperature, conductivity and E. coli colonies. The overall particulate debris were observed and sorted into microplastic and organic contaminants (leaves, vegetation and invertebrates) using light microscope. The samples were analyzed using wet peroxide oxidation (WPO) method to decompose any naturally occurring material. The largest amount of microplastics found throughout all streams, were in the form of fibers and the lowest in the form of beads. Microplastics structural makeup was then verified using an infrared spectroscopy (IR). E. coli was tested using an enzyme plating technique (ColiPlate™). All four sampled stream had unsafe levels of E. coli colonies for drinking water (>1MPNs) with an average of 124.5 MPNs/100 ml, and three out of the four having unsafe levels of E. Coli for recreational water (>100MPNs) with an average of the three being 158 MPNs/100 ml. Results of the study call for immediate action in protecting tributaries and other water resources from entry of microplastics.

443 Uptake and Retention of Microplastic Particles by the Daggerblade Grass Shrimp, Palaemonetes pugio

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The incidence of microplastics in marine environments has been increasing over the past several decades. The objective of this study was to characterize the size and shape dependent effects of microplastic particles (spheres, fibers and fragments) on the adult Daggerblade Grass Shrimp, Palaemonetes pugio. Grass shrimp were initially exposed to one of the seven fractions of plastic spheres (30, 35, 59, 75, 83, 116, and 165 µm), two size fractions of fragments (34 and 93 µm), and two size fractions of fibers (34 and 93 µm) at a concentration of 50,000 particles/L for three hours, then placed in microsphere-free water and monitored for survival, ingested and ventilated microplastics, and residence time in the gills and gut. Mortality ranged from 0-55%. Beads and fragments under 50 microns were not acutely toxicity. Those above 50 microns had mortalities ranging from 5-40%. Fibers were acutely toxic at both size fractions tested (34 and 93 mm) with mortalities at 55 and 35%, respectively. Shape of the microplastic particle did not influence mortality ($p=0.1547$). Mortality was significantly higher in the 83 micron spheres and 93 micron fibers than other sizes tested ($p< 0.001$). The average amount of particles ingested ranged from 2.3±1.7 particles/shrimp in the 93 micron fiber exposure to 28.8±26.4 particles/shrimp in the 75 micron sphere exposure. The number of particles found within the gills following the three hour exposure period ranged from 1.3±1.6 particles/shrimp in the 75 micron sphere exposure to 12.5±9.4 particles/shrimp in the 116 micron sphere exposure. Shape of the particle had a significant influence on the number of particles ingested by the shrimp, with spheres having the highest amount ingested ($p< 0.001$). The time for particles to be cleared from the gut of the shrimp ranged from 27-75 hours with an average at 43.0±13.8 hours. Within the gill chamber of the shrimp, the time for particles to be removed ranged from 27-45 hours with an average of 36.9±5.4 hours. Shape had no significant influence on clearance time in either the gut ($p=0.1625$) or the gills ($p=0.1545$). Results from this study suggest that microplastics in the environment of various size and shapes can have acute effects on shrimp.

444 Ingestion and rejection of microplastics by suspension-feeding bivalves: implications for exposure and environmental fate

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Suspension-feeding bivalves are exposed to a manifold of natural and anthropogenically-derived particles in the aquatic environment. Which particles are actually captured and ultimately ingested depends on several physicochemical and biological factors including limitations of the feeding structures and particle-selection capabilities of the species. In a series of experiments with blue mussels (Mytilus edulis) and eastern oysters (Crassostrea virginica) we examined the capture, selection and ingestion of several different types of microplastics with different shapes, sizes and surface properties (wettability, surface charge). Particles < 2 µm are captured at a low efficiency unless they are incorporated into marine aggregations. Large (> 100 µm) fibers and angular / spherical particles are captured at a high efficiency but are rejected in pseudofeces and thus ingested in low proportions. Particles between 10 and 20 µm are more likely ingested if they have more hydrophobic surfaces or possess surfaces with a lower charge (Zeta potential < ca. -8 mV). These results advance our understanding of the types of microplastics that are ingested by bivalves and those that remain in the environment. Developing physicochemical profiles of different types of microplastics to predict which are rejected versus ingested is important for assessing internal exposure and environmental fate.

445 Cellular accumulation of polystyrene particles in the Eastern oyster: dependence on size

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The global annual usage of plastics has increased dramatically over the last decade. Plastic polymers take on many forms with polystyrene being the fourth most common plastic material produced annually due to its many versatile applications. Consequently, there has been a coinciding increase in polystyrene wastes, much of which makes its way into waterways and oceanic habitats. While plastic debris has been shown to adversely affect many marine species as a result of ingestion and entanglement, less is known about the effects of plastic particles on marine invertebrates. We investigated the potential for particle uptake by hepatopancreas and gill cells in vitro and/or in vivo using fluorescent microscopy after exposure to 10ppb, 50ppb, and 100ppb of either 50nm or 3um fluorescent polystyrene particles. Epifluorescence microscopy indicated both 50nm and 3um particles were associated with both tissue types. More detailed studies using Deltavision Elite deconvolution microscopy revealed that the 50nm particles, but not the 3um particles, were intracellular. To assess the toxicity of the particles, lysosomal destabilization and lipid peroxidation were evaluated and the particles were found to have no effect on either. These results demonstrate that the Eastern oyster takes up polystyrene particles from the water column and that particles in the nano size range enter hepatopancreas and gill cells. Based on these short term studies plastic fragments in the nano size range are bioavailable to oysters and while no evidence of toxicity from polystyrene was observed, it is possible the plastic particles will cause long term stress to the animals by interfering with feeding processes.

446 Microplastic particle exposure and life-history traits of Chironomus riparius

W. Kunce, Uppsala Univ / Animal Ecology

Due to wide-spread detection in aquatic environments, microplastic particles are an emerging contaminant of concern. Research on the biological effects of microplastic ingestion in freshwater fauna is scarce. This two-part study investigated the impact of 1.0 µm and 10.0 µm fluorescent polyethylene spheres on life-history traits of the midge, Chironomus riparius. The impacts were assessed under restrained and unrestrained nutritional conditions as well as in combination with a low concentration of the commonly used pyrethroid pesticide, esfenvalerate. Sediment spiked with microplastic particles impacted both development time and survival of the midges with differences between the sexes; effects that were exasperated by food restriction but not esfenvalerate exposure.

447 Trophic transfer of microplastics and adsorbed contaminants

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Microplastics (MPs) are a widespread and ubiquitous contaminant in aquatic ecosystems. MPs are in the same size fraction as most sediment particles and microorganisms, allowing them to be easily ingested by a variety of organisms. Should ingested MPs have adsorbed organic contaminants, MPs may act as a source of environmental contaminants. The potential for MPs to alter the bioavailability of contaminants, and whether MPs and adsorbed contaminants can undergo trophic transfer has been investigated in very few studies. Polycyclic aromatic hydrocarbons (PAH) are a class of persistent organic pollutants that are ubiquitous in aquatic ecosystems. Fluoranthene (FLU) is one of the USEPA's 16 priority PAHs, and it's hydrophobic nature allows it to sorb easily to sediment and organic matter; FLU will likely sorb to the hydrophobic surfaces of most MPs. Freshwater amphipods exposed to FLU-contaminated polyethylene MPs resulted in the dose dependent uptake of both FLU and MPs during 10-d bioassays. FLU uptake via MP ingestion accounted for approximately 93% of the FLU body burden. To investigate the potential for MPs and adsorbed FLU to undergo trophic transfer, fathead minnows were trained to consume amphipods that had been previously exposed to FLU-contaminated MPs. The bile of exposed fathead minnows was analyzed for FLU by measuring bile fluorescence. Bile from fathead minnows that consumed amphipods previously exposed to FLU-contaminated MPs was 94% more fluorescent than that extracted from control organisms. Fathead minnows indirectly exposed to FLU via prey ingestion for 3 days resulted in the bioaccumulation of approximately 300 µg FLU/organism, demonstrating that trophic transfer is a pathway in which higher trophic level organisms can be exposed to MPs and associated contaminants. This study is one of the few that has quantified the bioaccumulation of MP-associated contaminants from living prey. Ongoing studies will demonstrate whether the ingestion of fathead minnows exposed to FLU-contaminated MPs by hybrid striped bass will result in increased bioavailability of FLU to larger predatory organisms.

From Phosphates to Food Webs: A Tribute to David Schindler's Legacy in Aquatic Sciences

448 David Schindler's legacy: Ecosystem-scale ecotoxicology

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One of Dr. David Schindler's greatest contributions to the field of environmental toxicology and chemistry is his pioneering application of whole-ecosystem experimentation to answer questions about the fate and effects of aquatic pollutants. At the Experimental Lakes Area (ELA) in northwestern Ontario, Dr. Schindler led ground-breaking studies in which contaminants were intentionally added to pristine headwater lakes. By simulating pollution in a controlled manner and at a realistic scale, these studies led to discoveries of food web interactions, long-term biogeochemical processes, and unexpected indirect effects that could not have been determined from smaller-scale studies. Whole-lake additions of nutrients and acids to ELA lakes during the 1960s and 70s not only advanced our scientific understanding of eutrophication and acidification, but were instrumental in changes in policy to combat these environmental problems. Since Dr. Schindler's departure from the ELA, his legacy has flourished. Over the last four decades, more than 50 whole-ecosystem experiments have been conducted at the ELA, including studies on mercury, synthetic estrogen, and nanosilver. In this presentation, we will highlight recent examples of whole-ecosystem contaminant-loading experiments to demonstrate how this approach to ecotoxicology is incredibly powerful and influential.

449 Whole-lake manipulations at the Experimental Lakes Area do not support the need for nitrogen control to reduce eutrophication in lakes

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In the 1960s and 1970s, whole-lake experiments undertaken at the Experimental Lakes Area (ELA) by David Schindler and his colleagues clearly demonstrated that cultural eutrophication was not caused by inputs of carbon (C) and that nutrient reduction efforts should focus on phosphorus (P). Considerable debate has persisted, however, about the need to regulate nitrogen (N) and several government agencies around the world are currently calling for dual control of both N and P. Over the 48-year history of the ELA, there have been 10 whole-lake experiments that involved additions of N, P, or N and P combined. Taken together, the results from these experiments provide no evidence that additions of N, either alone or in combination with P, result in substantially greater standing crops of algae in fertilized lakes. Recent data from Lake 227, which has been continuously fertilized since 1968, first with N and P and then only with P, provide no indication that reductions of N loading will mitigate eutrophication in lakes. Despite 25 years of high P loading without concomitant N additions, concentrations of total phytoplankton biomass and chlorophyll in Lake 227 have not declined. If anything, the reduction of N loading has adversely affected water quality by promoting the development of filamentous N-fixing algal blooms. The ELA data indicate that over-reliance on results obtained from small-scale studies, such as nutrient addition bottle assays, provides a poor indication of the long-term outcome of nutrient reduction strategies in natural lakes.

450 Recovery from Acidification: A Forty Year Experiment in the Killarney Park Area, Canada

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Killarney Park, a wilderness area of ultraclear lakes and white quartzite mountains located about 60km south of the giant Ni and Cu smelter in Sudbury, Canada, was one of the first places in North America where the link between atmospheric pollution, acidification of lakes, and loss of fish was clearly made. The severe damage to these once "pristine" lakes and landscapes, so treasured by artists, canoeists and fisherman, proved to be a significant match that ignited the regional, national and then international battles in the 1970s and 80s to reduce "acid rain" to acceptable levels. Regulatory agencies attempted to set targets of emission reduction that were tolerable to industry and were based on available technologies. Rarely, however, were the targets based on measured and modeled geochemical sensitivities (e.g. "Critical Load Models") of specific landscapes and lakes. Therefore in general we can view the emission reductions programs in North America very much as a large scale regional experiments, not unlike the whole lake acidification experiments that Dr. Schindler and his team conducted at L223 in the Experimental lakes Area, but conducted over a much longer time period. In this presentation I will review the history of acidification in the Killarney lakes and illustrate the variation of chemical and biological recovery in the key monitoring lakes. I will also show how the acidification monitoring program proved to be so valuable in detecting other environment changes (e.g. changing DOC and thermal structure, Ca decline, climate change impacts, invasive species, etc.) and will attempt to compare and contrast these findings from those of L223.

451 Following David Schindler's Lead: Understanding the Importance of Atmospheric Deposition of Polyaromatic Compounds in the Alberta Oil Sands Region

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Atmospheric deposition of contaminants originating from a variety of sources, including bitumen upgrading facilities and blowing dusts from landscape disturbances is of concern in the Athabasca oil sands region of northern Alberta, Canada. This issue was first examined in detail by the Schindler research group who sampled the winter snowpack at 28 locations along the Athabasca River and its tributaries in March 2008 and reported elevated deposition of polyaromatic compounds (PACs) and metals (Kelly et al PNAS 2009; 2010). Since 2011 we have collected snowpack samples annually in late winters at varying distances from the main developments to confirm and extend that work. PAC concentration and deposition declined exponentially with distance, with pyrenes, chrysenes and dibenzothiophenes (DBT) dominating the distribution within the first 50 km. The distribution of PACs was different between sites located close to upgraders and others located close to mining facilities. PAC loadings were correlated with heavy metals and water chemistry parameters, while wind direction and speed were not strong contributors to the variability observed. PAC mass deposition within the first 50 km was initially estimated by integrating the exponential decay function fitted through the data, using the same sites as Kelly et al in 2008. Total loadings were estimated to have increased 2-fold between 2008 (636 kg) and 2014 (1,367 kg), although the increase observed was not constant. Finally, kriging interpolation was applied to the same data and proved to be a more robust approach to estimate PAC mass deposition in the area when sampling sites were located in a gridded pattern over the landscape. Using kriging the PAC mass deposition for 2014 was estimated to be 1,968 kg, about 30% higher than assuming uniform decline from a central site near the upgrading facilities. Our second approach to examining atmospheric deposition was to reconstruct long-term terms of PACs using dated sediment cores from isolated lakes in the oil sands region. Results for PACs from twenty-eight lakes show that sites within 50 km of the upgrading facilities show a striking increase in petrogenic sources based on 8-fold increase in DBTs and a 3-fold increase in alkylated PACs compared with less than 2-fold increases in lakes from 60-185 km distant. These increases began in the 1970s coinciding with the start of the oil sands mining and point to the increasing importance of atmospheric deposition over time.

452 Building on David Schindler's legacy: Assessing impacts on lakes across spatial and temporal scales using lake sediments as archives

J.M. Blais, J. Korosi, J. Thienpont, Univ of Ottawa / Dept of Biology; J.P. Smol, Queens Univ / Biology

David Schindler's career showed what a profound impact aquatic science can have on public policy over a wide range of environmental issues, including acid rain, eutrophication, and the impacts of extractive resource development. This presentation builds on that legacy and summarizes recent developments in assessing the effects of multiple stressors from resource development on lake ecosystems using lake sediments as archives. We focus on integrating the principles of ecotoxicology with lake sediment analyses in our work to develop the field of "paleo-ecotoxicology". We argue that this framework is useful to test predictions from laboratory bioassays in natural ecosystems to assess biotic responses to contaminant exposure. Using the example of legacy contamination from gold mining in Yellowknife lakes, we examine how lake sediment cores can be used in an interdisciplinary context to enhance our understanding of the response of aquatic biota to stressors, and we demonstrate how this science has informed policy and management of this region.

453 Integration of Indigenous knowledge and western science in studies of northern ecotoxicology: lessons from David Schindler and other revolutionaries

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David Schindler's research legacy extends beyond cutting-edge science to include advocacy for environment-related rights, ethics, and knowledge of Indigenous peoples. Indigenous knowledge of water quality, fish health, fisheries, climate change, and cumulative effects is often overlooked or undervalued by western scientists and policy-makers because the two knowledge systems (western science, traditional knowledge) have different strengths, and because western scientists and policy-makers often believe that knowledge must be presented dispassionately; Indigenous peoples have an intergenerational emotional and spiritual bond with their environment that cannot be separated from their knowledge. In this presentation, we will use case studies to illustrate the strengths, opportunities, and pitfalls of collaborative projects that use both traditional knowledge and western science to investigate ecotoxicological questions. We will focus on Canada's North, where Indigenous people rely heavily on aquatic ecosystems for subsistence foods, drinking water, and spriritual and cultural purposes, and where jurisdictions are developing water-related policy and monitoring programs from a foundation of Indigenous values. Although much progress has yet to be made, it is obvious that the efforts and passion of David and countless others are having an effect on how research is conducted and policy is developed in Canada's North.

454 The influence of climatic trends and variability on fish growth and survival in freshwater lakes

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Though climate warming over the past half century is well documented, few datasets exist over a similar time frame with which to evaluate potential cimate effects on fishes in the absence of other potential stressors. At the IISD-Experimental Lakes Area (ELA) in Northwestern Ontario, mean annual air temperatures have increased by more than 1.5°C on average since 1969. Similarly, the ice-free period on Rawson Lake has increased by 17 days on average. Throughout this period, significant annual variation has also occurred, with mean annual temperature ranging nearly 5°C and ice free days by 47 days. Young-of-year fish make an excellent subject of study for evaluating climate effects on fish growth, for whom all surplus energy is reflected in somatic tissues. Additionally, overwinter survival of young-of-year in many fish species has been shown to be directly correlated with body size entering the winter. We investigated somatic growth patterns and associations with an index of overwinter survival in young-of-year fishes from reference lakes at the Experimental Lakes Area. Growth of Yellow Perch, Northern Redbelly Dace and White Sucker in their first year all demonstrate positive correlations with mean annual air temperature and growing degree days > 5C). Pearl Dace and Fathead Minnow young-of-year growth demonstrated a positive association with the number of mean annual precipitation, and Pearl Dace growth was also positively associated with the number of ice-free days. Variation in young-of-year fish growth in reference lakes due to climate variation was equivalent to or greater than that observed in lakes undergoing experimental manipulations. An initial assessment of overwinter survival did not appear to be linked to young-of-year body size. Associations of recruitment success with environmental conditions will also be presented.

455 Climate futures for temperate lakes: winter biogeochemistry and the vulnerability of lakes to change

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There is considerable concern regarding how climate change will impact lakes via factors including changing solute loads, increased temperature and altered stratification regimes. Anticipated effects include the potential for higher productivity, longer duration of benthic hypoxia, increased bloom risk, and key impacts on cold water fish habitat. Our understanding of, and ability to manage for changing conditions hinge on our understanding of the open water season. Yet we see rapid declines in periods of ice cover, and significant spatial and interannual variation in snow. The limited amount of research to date on under-ice limnology constrains our ability to predict consequences of a changing climate. Here, we assess observational data, coupled with rate measurements, and literature meta-analyses to assess key drivers of changing nutrient biogeochemistry under ice, and identify ecosystems where shorter ice cover duration is most likely to affect nutrient availability. We identify shallow, eutrophic and hypereutrophic lakes as the systems where winter increases in phosphorus are most likely to occur, with evidence that internal loading is the key driver of winter increases. We see key nitrogen processes of nitrification and denitrification as surprisingly active, despite cold water temperatures, yet showing high spatial variation among ice-covered ecosystems. Winter is not a quiescent period, but instead one where the processing of nutrients change. A shorter winter period may affect nutrient availability at the end of the winter, with the most marked impacts potentially occurring in shallow, nutrient rich ecosystems. While a shorter winter may actually lead to lower concentrations of phosphorus in some ecosystems, we need to understand the fate of phosphorus at ice-out, and develop an integrated view of climate impacts on lakes, that incorporates both impacts on summer, and the under-studied winter period.

Fate and Effects of Metals: Biogeochemical Perspective

456 Effects of Oxidation on Metal Bioavailability and Metal Release from In-place Sediments: A Modeling Perspective

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The presence of sulfides in sediments has been shown to play an important role in sequestering metals (e.g., Cd, Cu, Ni, Pb, Zn) and in limiting metal bioavailability. This effect has been considered in regulatory decision-making through static or "snap-shot" measures of simultaneously extractable metals (SEM) and acid volatile sulfide (AVS) ratios. A potential weakness in this approach is the oxidation of metal sulfides and associated changes in metal bioavailability that are likely to occur during oxidation of in-place sediments (e.g., due to changes in overlying water quality or reductions in sediment diagenesis activity). A reactive-transport model was therefore developed using the TICKET framework and was applied in evaluating metal bioavailability and metal release during oxidation of in-place sediment. TICKET model results showed that metal bioavailability and metal release to the overlying water are intricately linked to reduced iron and metal sulfide oxidation rates, precipitation of hydrous ferric oxide (HFO), formation of metal-sulfide complexes, and binding of metals to natural organic matter and newly precipitated HFO. The TICKET model was subsequently used in evaluating the potential for increased metal bioavailability and metal release based on the initial ratios of metal, AVS and iron at contaminated sediment sites.

457 Sediment characteristics affecting internal loading of arsenic in a prairie reservoir, Buffalo Pound Lake, SK, Canada

L.P. D'Silva, K. Liber, Univ of Saskatchewan / Toxicology Centre; H. Baulch, Univ of Saskatchewan / School of Environment and Sustainability; L.E. Doig, Univ of Saskatchewan / Toxicology Centre

Buffalo Pound Lake, SK, Canada, is a shallow eutrophic reservoir that has experienced an increased frequency and severity of algal blooms in recent years. Lake sediments can naturally contain and release large quantities of phosphorus (P) to associated overlying waters, and are possibly stimulating these algal blooms. In addition to P, Buffalo Pound Lake sediments naturally contain high concentrations of arsenic (As; 13.2 mg/kg). Arsenic is a geochemical P analogue, which may be released concurrent to P. Experiments using sediment cores collected from Buffalo Pound Lake identified environmentally relevant mechanisms (warm temperatures, low dissolved oxygen (DO) concentrations, and alkaline pH (9.3)) in lake water that significantly increase As efflux from bottom sediment. The objectives of this study component were to determine summertime in situ dissolved As concentrations in the lake, mechanisms affecting in situ As remobilization, and differences in sediment As content and geochemistry among sites. Water samples and sediment cores were collected from Buffalo Pound Lake biweekly. Water samples were analyzed for dissolved metal(oid) s (As, aluminum (Al), iron (Fe), manganese (Mn)), temperature, DO, and pH. Sediment cores were sectioned and analyzed for porewater metal(oid) (As, Al, Fe, and Mn) content. Bulk field sediment was analyzed for total Al and Fe content, total organic content, and particle size distribution. Periods of warm temperatures and low DO concentrations were associated with greater As concentrations in bottom waters. One site in the down-reservoir region displayed lower efflux of As from sediment. This site had significantly elevated Al content relative to the other sites studied. Aluminum can act as a redox-insensitive sorbent of As, which likely mitigated reduction-induced release of As from sediment at this site. Arsenic concentrations in surface waters ranged from 3–9 µg/L, with several samples exceeding federal water quality guidelines (5 µg/L) for the long-term protection of aquatic life. Therefore, Buffalo Pound Lake sediment can release As to overlying waters during low DO and high temperatures periods. Additional work will investigate the availability of As during summer As release.

458 Arsenic sorption to bacteriogenic iron oxides (BIOS)

M. Moriarty, Univ of Ottawa; D. Fortin, Univ of Ottawa / Earth and Environmental Sciences

Bacteriogenic iron oxides (BIOS) form at water-rock interfaces, wherever anoxic waters rich in iron emerge, such as mine seep environments where arsenic concentrations may be elevated. BIOS are (mostly) amorphous iron oxides that contain large amounts of organic matter, rich in polysaccharides, and are less resistant to bacterial reduction than organic matter-free iron. They form quickly and are important sorbents for cationic metal ions due to the presence of negatively charged organic matter and high surface area. The sorption capacity of amorphous iron oxides appears to be constant for both the neutral As(III) and the anionic As(V) for a wide range of concentrations of organic content at circumneutral pH. We have built a diffusion growth chamber to model an iron seep environment in order to investigate the behaviour of arsenic, its redox transformations in water and resulting solids and the short-term stability of immobilized arsenic in mine seep environments with iron oxidizing bacteria present with varying dissolved Fe(II) concentrations, dissolved and solid organic matter and sulfate levels. Laboratory experiments are compared with a wide range of field data. This talk will discuss how the presence of bacteria in iron rich environments affects the speciation and mobility of arsenic.

459 Rare earth element sorption: lessons learned from field collected biofilm data

L. Ashby, Univ of Ottawa / Earth Sciences; D. Fortin, Univ of Ottawa / Dept of Earth and Environmental Sciences

The partitioning of metals from liquid to solid phase has been of interest for remediation of toxic metals for many years. Rare earth elements (REEs), historically not considered toxic metals, have received little attention regarding mechanisms of adsorption to the solid phase. Metal-oxides, organic matter and bacteria are all known to play important roles in removing REEs from solution, but their relative contributions and importance are not fully understood. Understanding biotic and abiotic sorption has both environmental and industrial implications. We examined the sorption of REEs to biofilms, which are a mixture of bacterial cells, oxyhydroxides, metal precipitates, and bacterially-derived organic matter, as these materials appear to be very good sorbents for REEs. This study compared field collected water and biofilm chemistry collected from a total of 8 locations (40+ sites) in North and South America, downstream of mining-affected areas (Au-Ag, Cu-Au-Ag and coal deposits), with 2 control locations (6 sites) and modelled data. Partitioning coefficients ranging from 1 to 1E6 were found within the dataset, and at least two binding mechanisms are suggested. Field-collected biofilm chemistries were compared with modelled biofilm chemistries predicted using a surface complexation model.

460 Role of Natural Organic Matter on Rare Earth Elements Speciation and Bioavailability with Chlamydomonas reinhardtii

J. Rowell, K. Wilkinson, Univ of Montreal / Dept of Chemistry

As technological interest for rare earth elements (REE) is growing, it is becoming more important to assess their potential environmental impact. Indeed, specific knowledge is required about the behaviour of these metals in rivers and soils, more precisely with respect to the factors that influence their speciation and bioavailability. Based upon models such as the Biotic Ligand Model (BLM) that have been successful for predicting the effects of divalent metals, the complexation of metal ions by natural organic matter is predicted to reduce the free ion concentration and simultaneously decrease metal bioavailability for aquatic organisms. Nonetheless, recent studies from our laboratory using simple organic ligands have shown that REE biouptake is higher than predicted and that internalization fluxes are higher than predicted in presence of fulvic acid. Thus, it becomes extremely relevant to carefully determine REE speciation in natural waters, especially in the presence of natural organic matter, in order to predict their bioavailability. In this study, a dynamic ion-exchange technique was used to determine free ion concentrations and in parallel, biouptake experiments were performed to measure the internalization flux of REE in algae. Samarium (Sm), an intermediate lanthanide, and the unicellular green alga Chlamydomonas reinhardtii were employed, and results were compared with modelling efforts in order to better understand the important factors leading to REE bioavailability.

461 Subcellular partitioning profiles and molecular biomarkers in in-situ exposure clam and their associations with metal speciation as measured by DGT

Z. Wang, Key Laboratory of Urban Environment and Health; C. Yan, Insitute of Urban Environment, Chinese Academy of Sciences

Due to the complexity of heterogeneous sediment, the chemical speciation and mobility of metals were in dynamic conditions rather than steady states, resulting in high variability of bioavailability. Chemical analyses in traditional tests had disadvantages of inaccuracy for contamination characteristics or risk assessments, representing a worst-case scenario in terms of complex biotic and abiotic exposure dynamics. In this presentation, relevant scientific issues regarding the impacts on subcellular distribution and molecular biomarkers with special emphasis on their associations with various metal species were investigated through powerful experimental designs and field-based manipulations of in-situ exposure and kinetic DGT approach. Understanding the degree of pollution and geochemical characteristics of sediment matrix, in-situ testing chambers were deployed combining the DGT and caged clam R. philippinarum, and the dynamic exchanges, translocation and mobilization were then clarified among the interfaces of particles and pore-water in sediments through measuring dynamic parameters and resultant induced fluxes in exposed organism. Integrating a wide battery of biomarkers, dynamic changing processes were assessed, and their interactions were established between exposure and biological effects. Furthermore, variations of transcript expression of functional genes in contaminant-specific biomarkers were obtained through high throughput oligo-DNA Microarray and quantitative reverse transcription polymerase chain reaction (Q-RT-PCR) in order to elucidate the mechanistic understanding of molecular effects metals induced. Simultaneously, the approach of in-situ evaluation was established considering the consistency of framework as protocols among sediment chemistry, contaminant bioavailability and adverse effect, which significantly improve accuracy and ecological relevancy in complex exposure situation and thus provide a robust tool to support more comprehensive processes of sediment risk assessment. Overall, the associations not only revealed the fates of accumulated metals, but scientifically favored an improved understanding of toxic effects in response to subcellular level and metal speciation, supporting the focus of metabolic availability on the intracellular processes or events occurring within organism in coastal biogeochemistry. (The author acknowledge financial support by Grant No. 21377125/B070403 from National Nature Science Foundation of China)

462 Bioaccumulation of Cu and Pb by fathead minnow and yellow lampmussel: Evaluation of DGT technique for predicting uptake of metal mixtures

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Dissolved metal bioavailability in natural waters is strongly linked to metal speciation. Diffusive gradients in thin-films (DGT) were designed as a passive diffusion, in-situ sampling device with the goal of providing a rapid method for determining the free ions and labile complexes of total dissolved metal in surface waters, i.e. the bioavailable fraction. In this study, DGT were assessed for their predictive capability of fathead minnow and yellow lampmussel sublethal bioaccumulation in copper (Cu) and lead (Pb) mixed metal exposures. Nine treatments with a matrix of three Cu concentrations (0, 15, 25 µg L^{-1}) and three Pb concentrations (0, 30, 50 µg L^{-1}) were utilized. Exposures were coupled, with organisms and DGT exposed together in tanks, water only, no feeding for six day durations. Copper measured in fathead minnow, yellow lampmussel, and by DGT was found to not be influenced by lead treatment, whereas accumulated lead was impacted by the interaction of Cu treatment and Pb treatment. DGT significantly correlated with fathead minnow accumulated Cu and Pb. Yellow lampmussel bioaccumulated metal were not as highly linearly correlated with DGT. In addition, DGT was determined to better predict aquatic organism bioaccumulation of copper than the inorganic Cu fraction modeled by the Biotic Ligand Model speciation component.

463 Could climate change affect metal pollution in estuaries?

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Estuaries are vulnerable to metal pollution and climate change consequences (sea level rise, shifts in river discharge, and coastal squeeze). While current metal sources are problematic, studies suggest that legacy metal on sediments might also be bioavailable. We measured metal concentration in estuarine sediments, mobility (leaching from sediments to water phase), and developed a state-of-the-art hydrodynamic-geochemical model for metal fate in an idealized estuary to infer the potential impacts of climate change. Surface (5cm) and subsurface (up to 2m) metal distribution and physicochemistry of sediments (N~600) in the tidal flats of the former Newlands Landfill (Thames Estuary, UK) were analyzed. Metal concentrations there were patchy and showing little continuity to the proximity of the landfill. In situ metals provided stronger evidence of chemical mobility as major actor on metal concentrations, e.g. Cu was correlated to organic matter, while Sr was influenced by Ca, and Co was determined by pH. Importance of chemical mobility was confirmed when we exposed (24h) the upper 2cm of these surface sediments (N~96) to environmentally relevant gradients of salinity, redox, and pH to determine the leaching of metals from those sediments to water. Under certain conditions (e.g. low salinity, negative redox and pH~ 6.5 for Fe^{++}), metal leached increased ~ 1000-fold. Using the coupled hydrodynamic-metal fate model (Delft3D), we simulated the fate of particulate and dissolved metals in water column and sediments under various climate change scenarios (i.e. 3 coastal squeeze scenarios, 3 altered discharges and 2 tidal conditions). The model results on total (particulate + dissolved) metal concentration in water column imply that salinity fronts and tides are critical for metal immobilization and increasing metal residence time, while remobilization was mainly occurring on tidal flats due to constant settling/reworking of particles. This caused a non-conservative behavior of metals. Thus, leaching experiments pointed that climate change could cause metal mobilization from sediments if it decreases pH, or changes redox conditions and sediment salinity. Also, our model suggests that climate change could increase metal pollution if salinity fronts and maxima turbidity migrate upstream implying higher residence time of metals within the estuary, or changes on tidal flats by coastal squeeze cause higher sediment reworking or residence time of suspended sediments.

Demonstrated Remediation Technologies Addressing Contaminated Soil, Sediment and Water

464 Enhanced degradation of benzo[a]pyrene in coal tar contaminated soils using biodiesel

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The biodegradation of the potent carcinogen, benzo[a]pyrene (BaP), and other priority pollutants was investigated in un-weathered coal tar contaminated soil over a period of 150 days. Results from laboratory microcosm experiments showed that after 60 days, the concentrations of BaP were significantly reduced by 81%, in the biodiesel amended samples compared to the 26% and 34% depletion in the control and nutrient-only amended microcosms, respectively. A stepwise treatment approach also revealed a higher reduction in BaP (98%) using biodiesel. Toxicity assays showed that biodiesel amended microcosms stimulated phosphatase enzyme activity and exhibited a lower toxic response to Microtox Vibrio fischeri. The enhanced removal of carcinogenic PAH and the reduced toxicity observed in soil after biodiesel amendment, indicates that this bioremediation technique has potential for full scale field trials.

465 From bioavailability science to soil bioremediation: Sustainable stimulation of biological degradation for enhanced removal of PAHs

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Polycyclic aromatic hydrocarbons (PAHs) are the best representatives of chemicals for which specific limitations in bioremediation exist due to low bioavailability. The residual concentrations of the PAHs after bioremediation are crucial because they may limit the use of the area after treatment, or land use might not even be possible if the residual concentrations do not meet the legal requirements. For bioavailability to be incorporated into soil bioremediation, three questions must be addressed: (1) how is "bioavailability" defined? (2) how should it be measured? and (3) is it possible to increase bioavailability but not environmental risk of the pollutants? Over the last 30 years, numerous publications have discussed the concepts and definitions of bioavailability of organic chemicals (Environ. Sci. Technol. 49:10255-10264, 2015). The main schools of thought consider bioavailability (focusing on the aqueous or dissolved contaminant), bioaccessibility (incorporating the rapidly desorbing contaminant in the exposure), and chemical activity (determining the potential of the dissolved contaminant for biological effects). These concepts are the basis for different methodologies (desorption extraction, passive sampling and biological tests) and mechanistic studies that consider the different processes that are involved (contaminant soil/sediment interactions, transport and passage across cell membrane, and biological responses such as toxic effects or biodegradation). Our group has proposed different ways to operate at different levels on these processes, in the context of biodegradation of PAHs, for a better bioremediation performance in risk reduction. The approach is relevant because in some circumstances bioremediation may even increase risk of PAHs.

466 Enhanced anaerobic biodegradation of petroleum hydrocarbons in sediments and water using bioelectrochemical systems (BES)

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Biodegradation rates of organic contaminants can be influenced by many factors including nutrient and terminal electron acceptor (TEA) availability. While nutrient addition can address the former issue, maintaining an aerobic environment that maximizes microbial activity can be costly in aqueous environments or nearly impossible in saturated soils and sediments. Once concentrations of more thermodynamically favorable TEAs like oxygen or nitrate are exhausted, facultative microbes must rely on less favorable TEAs such as sulfate, which ultimately reduce biodegradation rates. In this presentation we will discuss data from our research that indicates bioelectrochemical systems (BES) can enhance biodegradation of organic contaminants in anaerobic environments by providing microbes with an inexhaustible, solid state electrode that can be utilized as a TEA. The basic design of this system includes an electrically conductive anode that is placed in the anaerobic environment (water or saturated sediment), which is connected to a cathode that is in contact with oxygen. Microbes breaking down organic contaminants like hydrocarbons can pass electrons to the anode, which flow through a circuit to the cathode where they are used in the reduction of oxygen (producing water at the cathode). We will present and discuss hydrocarbon degradation rates in anaerobic environments, which were over 2.5 or 12 times higher using BES compared to controls in water or sediments, respectively. We will also discuss 16S rRNA gene sequencing results that characterize microbial consortia utilizing the solid state anode in some of these systems.

468 Use of Excretory Halophytes to Remediate a Salt-Impacted Site

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Salt-impacted soils are an increasing environmental issue affecting global soil quality and plant productivity. Soils can become salinized via both natural and anthropogenic activities. A site located in Bath, ON, Canada was contaminated with cement kiln dust (CKD), a fine-grained, solid, highly saline waste product. CKD was landfilled at the site over a 30-year period from 1973 to 2003 resulting in a wasteland of 1 kilometer squared that is largely devoid of vegetation. Due to the water soluble nature of the predominant potassium and chloride ions, phytoremediation techniques were investigated. In previous work, Phragmites australis (a resident invasive species) was found to accumulate large quantities of K^+ and Cl^-, while two native, halophytic grasses, Distichlis spicata and Spartina pectinata were identified as having potential to remediate the soil via excretion and subsequent haloconduction. During this process, salt is transported into plant biomass, excreted through specialized salt glands onto the plant leaf surfaces, and then dispersed by the wind. The goal of the present research is to determine whether haloconduction could be a viable remediation strategy at the site (as an alternative to other remediation techniques). Analytical work using X-ray dispersive scanning electron microscopy is being carried out to identify chemical and physical properties of the salt crystals. Salt collection trials are ongoing both in the field and under controlled conditions in the greenhouse in order to model salt dispersion of the two species over time. Preliminary results will be presented, as well as a discussion of how this information could be used to model the transport of salts over space and time.

469 Integrated Restoration/Remediation of a Mercury Contaminated River

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Historically released mercury (Hg) accumulated in depositional areas along the banks of the South River, Virginia. Natural erosion of river bank soils with elevated Hg concentrations is a primary mechanism for legacy Hg loading to the aquatic ecosystem. As part of initial measures under the regulatory authority of the Resource Conservation and Recovery Act (RCRA) Corrective Action, river banks are being remediated in an upstream-to-downstream sequence within an adaptive management framework. Initial review of conceptual remediation designs by stakeholders, including landowners and wildlife agencies, raised concerns regarding potential impacts to riparian and upland habitats (e.g., mature trees). The final designs strike a balance between the remedial objectives of RCRA and stakeholder objectives of maintaining riparian and near-bank aquatic habitat functions, improving access to the river for recreational activities, and minimizing disruption. Based on the outcome of collaborative design discussions between the involved parties, optimization and balancing of these objectives can be best achieved by focused soil removal actions in localized banks that contribute disproportionately to Hg loading. Less invasive enhanced vegetative stabilization designs have been developed for those banks that contribute relatively smaller Hg loading. This presentation will describe the collaborative approach and key consensus design elements applied to the various banks.

470 Altering Estuarine Sediment Transport Dynamics to Reduce Ecological Exposures: a Novel Form of Risk Management

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Conventional methods of contaminated sediment remediation include monitored natural recovery, in-situ capping, and/or dredging. However, coastal and estuarine sites often pose unique challenges to the conventional remediation paradigm, which can undermine long-term effectiveness, delay site closure, and increase monetary and non-monetary costs. As a novel form of risk management, a “living shoreline” pilot project was constructed at a former gun club on the Housatonic River Estuary in Stratford, Connecticut. The site was operated as a trap and skeet shooting facility from 1926 to 1986, which resulted in the discharge of lead shot into surrounding waters and sediments. Large-scale remediation occurred from 2000 to 2001, and consisted of excavating the intertidal and nearshore subtidal zones and extracting lead shot using a mineral jig. Historically, the remedial area was a depositional environment; however, since remediation, the site has been subject to substantial shoreline erosion and associated changes in shot densities. The living shoreline was designed to dissipate wave energy and associated scour, and disrupt long-shore sediment transport to reduce ecological exposure. Results have been evaluated in the context of a Before-After-Control-Impact statistical framework. Bathymetric surveys show that the living shoreline is reducing erosion and increasing fine-grained sediment deposition.

471 Batch Adsorption Studies on the Use of Sorghum Husk for Treatment of Heavy Metal Contaminated Wastewater

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Contamination of environment by heavy metals has become a major concern worldwide. Therefore, it becomes imperative to explore a cost effective and efficient alternative method of removing heavy metals from polluted soils and waste water. In this study, the adsorption behavior of Sorghum husk, a low-cost adsorbent, with respect to Pb^{+2} and Cd^{2+} ions, has been studied in order to consider its application to the treatment of wastewater. The batch method was employed: parameters such as pH, contact time, particle size and initial metal concentration were studied. The influence of the pH of the metal ion solutions on the uptake levels of the metal ions by the adsorbent used was carried out between pH 3 and 8. The optimum pH for Pb^{+2} and Cd^{+2} removals by sorghum husk were 6 and 5 respectively. An equilibrium time of 80min was required for the adsorption of Pb^{+2} and 60min for Cd^{+2} ions onto sorghum husk. Percentage sorption of Pb^{+2} increased from 21 to 80% and that of Cd^{+2} from 15 to 55% as adsorbent dose was increased from 0.1 to 1g while adsorption capacity decreased from 14 to 5.3mg/g for Pb^{+2} and 32.5 to 9.3 mg/g for Cd^{+2} with increase concentration. Percentage removal of Pb^{+2} increased from 24.8 to 93.7% while that of Cd^{+2} from 22.4 to 91.6 % with decrease in adsorbent particle size from 1000μm to 100μm. The uptake capacity of the adsorbent increased from 6.5 to 12.4 mg/g for Pb^{+2} and 2.1 to 5.3mg/g for Cd^{+2} as the initial metal ion concentration was raised from 50 to 200mg/L. When two isotherms, the Langmuir and Freundlich models, were applied to the experimental data, the best fit was obtained with the Langmuir model. The results showed that sorghum husk has the potential to remove cationic heavy metal species from industrial wastewater.

Assessing Contaminant Effects in Multi-Stress Ecosystems – Part 2

472 A rapid screening approach for distinguishing multiple stressors in souther California streams

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Over 100 biologically impaired stream segments have been identified in southern California, all of which could involve multiple stressors including ion imbalance, pesticides, metals, nutrients, and others. We developed a rapid screening approach that can identify the major stressor(s) as well as help prioritize restoration efforts. The approach combines several region-specific tools including species tolerance information and species sensitivity distributions for commonly encountered pollutants and an innovative tool for identifying distributions of stressors for comparison. We developed a correspondence table between land cover and candidate causes for the region, which is used to refine the list of potential stressors based on land use and other mapped information. To further refine the likely stressor(s), we performed a cluster analysis to identify groups of sites with similar natural characteristics, such as gradient, flow, climate, and underlying soil and geology, and similar inflexible anthropogenic characteristics such as land cover. Using benthic macroinvertebrate data from 374 sites and 835 samples we identified six clusters based on 33 abiotic variables. Each cluster had a different characteristic distribution of the 33 variables enabling separation of stressors when compared against impaired site data. Our approach was able to separate stressors using several pilot sites in the region.

473 Integrating Contaminant and Other Stressors in an Ecological Risk Assessment of an Urban Waterway

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Assessing ecological risk at urban sites is challenging due to the presence of multiple contaminants and other stressors, including habitat degradation and low dissolved oxygen levels due to high organic point source discharges. The ecological risk assessment conducted as part of the Newtown Creek Remedial Investigation used multiple lines of evidence to identify contaminants of potential ecological concern, and included an evaluation of non-contaminant stressors. Sediment bioassays showed clear spatial differences in toxicity when compared to a reference envelope. Synoptic measurements of porewater contaminants using solid-phase microextraction fibers and mini-peepers indicate that toxicity is likely due to polycyclic aromatic hydrocarbon (34), with some contribution from metals. Test results appear to be confounded by other stressors for stations close to some of the large municipal point sources, including combined sewer overflows. Benthic community data from these locations scored below those in the reference areas during the summer months when dissolved oxygen fell below the 3-milligram-per-liter New York State threshold. Exposure estimates for wildlife were site-specific, based on survey data for site use, foraging activity, and prey type, as well as tissue chemistry in prey. Risks to wildlife were low, and the spatial distribution was similar to, and therefore supported, the results of sediment bioassays. Habitat surveys showed that 99% of the site shoreline comprises bulkhead materials (concrete, metal, wood), riprap, and rock. In contrast, for three of the four reference areas, only 6 to 27% of the shoreline is developed. This is reflected by higher species richness for aquatic-dependent birds in the reference areas, which provide habitat and refuge for foraging along the shoreline. For fish and crabs, species richness and diversity were found to be higher in the reference areas; however, these differences were attributed to differences in salinity. Overall, this risk assessment demonstrates that other stressors have an important influence on the habitat and biological communities of the site.

474 Phenotypic characterization of urban runoff toxicity in juvenile coho salmon, Oncorhynchus kisutch

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Urban runoff is a leading source of nonpoint pollution due to its ability to quickly mobilize a diverse mixture of contaminants. A decade of field observations near Seattle, Washington correlated acute die-offs of wild adult coho to urban runoff exposure. Ultimately, this study intends to identify if juvenile coho are similarly affected by urban runoff, characterize the behavioral response in juveniles, and investigate the physiological mechanisms causing mortality. In a series of experiments, juvenile coho were exposed to collected urban runoff or control water for 4-6 hours. Behavioral response was monitored using behavioral data acquisition software and response was characterized using a categorical scale. Observable symptoms included increased surface activity, gaping, hyperactivity, loss in equilibrium, and immobility. To understand the physiological mechanism of toxicity, we formed two main hypotheses: 1) urban runoff causes histotoxic hypoxia, the inability of oxygen uptake by the tissues, through mitochondrial enzyme dysfunction, and 2) urban runoff causes ischemia, localized reductions in blood flow due to coagulation, preventing oxygen delivery to the cells. For this study juvenile coho were exposed to urban runoff or control water and sampled in a time series throughout the exposure. Blood was analyzed for evidence of metabolic disruption and coagulation using a point-of-care blood analysis tool, clotting time assays, and blood smears. Initial blood analyses showed significant changes between control and urban runoff exposed juveniles, suggesting metabolic acidosis and increased coagulation in exposed fish. Further analyses are needed to explore the specific timeline for each physiological mechanism to confidently pinpoint the cause of death.

475 Environmental Exposure to an Urban Wastewater Effluent: Effects on the Energetic Metabolism of Northern Pike

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Municipal wastewater effluents are complex mixtures of chemical and biological compounds that can impact aquatic organisms from receiving ecosystems. Adverse effects may occur at several levels of the biological organization. The aim of this study was to assess the effects of a major municipal wastewater effluent (Montreal, QC, Canada) on the energetic metabolism of environmentally exposed northern pike (Esox Lucius) using a multi-level approach. We hypothesized that effluent exposure is associated with greater halogenated flame retardant (HFR) concentrations in liver that are related to changes in thyroid status of fish and transcription level of genes involved in transport, synthesis, and metabolism of fatty acids. A total of 50 pikes were collected in 2014-2015 upstream and downstream of the point of discharge in the St. Lawrence River of Montreal's primary wastewater treatment plant (WWTP). Plasma levels of total and free triiodothyronine (T_3), percentages of total lipids (liver), body condition index (Fulton) and stable isotope signatures of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) (plasma, liver, and muscle) were measured and compared to hepatic concentrations of 34 polybromodiphenylethers (PBDE) congeners and 12 emerging HFRs. Concentrations of liver Σ_{34}PBDE were approximately 4-fold greater in pike collected downstream of the WWTP and thus confirmed an important effluent exposure at this site. General $\delta^{13}C$ and $\delta^{15}N$ profiles in pike tissues corroborated effluent exposure at this site. Moreover, effluent exposure was associated with a significant increase (40%) in total lipids in fish liver and a tendency for increasing free T_3 levels and free T_3/total T_3 level ratios in plasma of females. No significant difference in body condition index was observed between sites. Results suggest that the energetic metabolism of this predator fish may be affected by the chronic exposure to the effluent. Ongoing work that will be presented at the conference will include hepatic peroxisomal acyl-coA oxydase activity (ACOX) and transcription levels of l-fabp, fasn, pparγ and acox genes implicated in hepatic transport, synthesis and metabolism of fatty acids in the liver. These findings will contribute to a better understanding of the mechanisms of toxicity associated with exposure to effluents.

476 Temperature and photoperiod-dependent sex determination in branchiopod crustaceans is modulated by the NMDA receptor antagonist MK-801

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Branchiopod crustaceans are subject to environmental sex determination whereby the introduction of males into a population denotes the switch from asexual parthenogenetic reproduction to sexual reproduction. Sexual reproduction is initiated by environmental signals that cue the onset of adverse conditions, and diapausing eggs are produced in order to reestablish populations once favorable conditions resume. We determined the environmental cues necessary for male production in Daphnia magna and D. pulex by examining photoperiod (long day, 16 hrs and short day, 10 hrs) and temperature (16^o, 18^o, 20 and 22^o C). Additionally, we investigated the role of the NMDA receptor in male production with the NMDA receptor antagonist MK-801. Both species produced negligible or no male offspring when subjected to long day length regardless of temperature. D. pulex produced male offspring under the short day length at all four temperatures, with colder temperatures significantly increasing the proportion of males produced. D. magna alsoproduced male offspring under the short day length but with colder temperatures significantly decreasing the proportion of males produced. In all cases, male sex determination of offspring varied significantly among the first six broods where some broods appeared to be responsive to environmental cues and other broods were not. The NMDA receptor antagonist MK-801 increased male production in both species in broods where aged-matched control daphnids produced broods that were predominantly female, but reduced male production when control broods were predominantly male. Results suggest that daphnid male sex determination is triggered by a combination of photoperiodic and temperature cues that suppress the NMDA receptor. We propose that when the NMDA receptor is not actively suppressed by environmental cues, conditioning the organisms to produce female offspring, MK-801 functions as an antagonist resulting in increased male sex determination. However, when the NMDA receptor is actively suppressed by environmental cues, conditioning the organisms to produce male offspring, MK-801 functions as a weak agonist to decrease male sex determination of offspring. Thus, environmental contaminants interacting with the NMDA receptor may alter male production with adverse consequences on the spatial and temporal maintenance of these important primary consumers.

477 Evaluating arsenic additions and future trends in a complex ecosystem

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A case study was carried out in which a suite of air quality and watershed models was applied to track the ecosystem contributions of arsenic (As) emissions from three coal-fired power plants (CFPPs) located in the San Juan River basin in the Four Corners region of the American Southwest. Site and meteorological data from the period 1986-2012 were used to project air quality conditions and ecosystem impacts to the year 2074 using randomized sampling techniques. The arsenic depositing from the atmosphere originates from a variety of local, regional, and global sources. Lacking source-specific inventories, background arsenic was parameterized by application of background atmospheric concentrations. The watershed response to this deposition is a function of transport and transformation processes within the land and surface and ground waters. Because of the large storage capacity of soils, changes in the watershed due to changes in atmospheric inputs may take many years to become evident. Atmospheric arsenic was modeled using a nested modeling system employing the EPRI CMAQ-APT (national and regional) model. For the Four Corners region of the United States, where large-scale point source inventories of arsenic are lacking, power plant contributions were also quite small in comparison to background atmospheric concentrations. The WARMF watershed model integrated atmospheric deposition, soil-content inputs of arsenic, and direct discharge contributions of the substance to derive fluxes of arsenic for the soil and surface water constituents. The model was used to simulate watershed response to scenarios of future atmospheric deposition out to the year 2074. WARMF simulations showed the contribution of the primary power plant to surface water arsenic concentration was less than the 0.01% discernible by the model. Results from this study of arsenic, a multimedia toxic, were used to help guide research on potential health or ecosystem impacts in the region, including several ecological risk assessments at finer scale. Federal land management agencies in the region used the study results in connection with review of the power plants' environmental impact statements (EISs) and to gauge compliance with the Endangered Species Act (ESA).

478 Effects of Salinity on Oil Spill Dispersant Toxicity in Estuarine Organisms

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Chemical dispersants can be a useful tool to mitigate oil spills, but the potential risks to sensitive estuarine species should be carefully considered. To improve the decision making process, more information is needed regarding the effects of oil spill dispersants on the health of coastal ecosystems under variable environmental conditions such as salinity. The two oil dispersants used in this study were Corexit ® 9500 and Finasol ® OSR 52. Corexit ® 9500 was the primary dispersant used during the 2010 Deepwater Horizon oil spill event, while Finasol® OSR 52 is another dispersant approved for oil spill response in the U.S., yet considerably less is known regarding its toxicity to estuarine species. The grass shrimp, Palaemonetes pugio, and the mud snail, Ilyanassa obsoleta, were selected as common estuarine species that are tolerant of a wide salinity range. Adult and larval life stages were tested with each dispersant at different salinities, ranging from 5ppt to 30ppt. Median acute lethal toxicity thresholds and sublethal biomarker responses were determined. The toxicity of both dispersants was significantly influenced by salinity, with greatest toxicity observed at the lowest salinity tested. Larval life stages were significantly more sensitive than adults to both dispersants, and both life stages were significantly more sensitive to Finasol than to Corexit. These data will assist environmental managers in making informed decisions regarding dispersant use in future oil spills.

479 Multibiomarker evaluation of pollutant effects in Atlantic stingray (Dasyatis sabina) populations in Florida's St. Johns River

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The goal of this study was to examine the potential health effects of polychlorinated biphenyl (PCB) and polycyclic aromatic hydrocarbon (PAH) exposure on Atlantic stingray populations in Florida's St. Johns River (SJR). Special emphasis was placed on identifying PAH- and/or PCB-related effects in stingrays from areas of the lower SJR basin that have been shown to possess elevated levels of these compounds, as well as characterizing baseline levels of pollutant exposure in other areas that may be subjected to dredging in the near future, potentially resuspending and redistributing contaminated sediments and increasing pollutant-associated effects. To accomplish this, we measured PCB and PAH biomarker levels in stingrays collected from contaminated sites and reference locations. We specifically examined the biomarkers cytochrome P4501a1 (CYP1a1), a Phase I detoxification enzyme; glutathione-S-transferase (GST), a Phase II detoxification enzyme; uridine diphosphate glucuronosyltransferase (UGT), a Phase II detoxifiocation enzyme; fluorescent aromatic compounds (FACs), PAH bile metabolites; lipid peroxidation (LPO), cell membrane damage; and thyroid epithelial height (TEH), an assessment of endocrine disruption-induced hypothyroidism. Enzymatic activity of CYP1a1, GST, and UGT was measured using an EROD assay, a commercially available GST Assay Kit, and a 4-methylumbelliferone assay, respectively. LPO was measured with an OxiSelect TBARS Assay Kit. FACs were measured using fixed wavelength fluorescence and TEH was analyzed histologically. Biomarker levels of individuals collected from contaminated sites were compared to individuals collected from reference sites. The data suggest that pollutant biomarker levels in SJR stingrays did not differ significantly from those measured in individuals from reference locations. However, the close proximity of high biomarker levels to known contaminated areas suggests continuous exposure.

Contaminant Flux Across Environmental Compartments and Implications for Global Distribution

480 Recent developments in methodologies for determining sediment to water fluxes of hydrophobic organic contaminants in situ

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Processes occurring at the sediment-water interface affect abiotic transfer of hydrophobic organic contaminants to overlying water, as well as contaminant exposure to benthic fauna and contaminant migration via the aquatic food chain. Organic contaminant transfer at the sediment-water interface is dependent on freely dissolved aqueous concentrations. This has led to an increased focus and research to determine in-situ the freely dissolved concentration gradients and the contaminant mass transfer across the sediment-water interface. Results from the use of several innovative methods to determine sediment-to-water fluxes in situ will be presented: 1) Use of a closed flux chamber to determine sediment-to-water fluxes before and after capping and activated carbon amendment 2) A novel flow through flux chamber enabling measurement of flux while renewing the water inside the chamber in order to supply oxygen to the benthic fauna. This enables measurement of sediment to water fluxes with conditions sustaining benthic life and therefore includes the effect of bioturbation on the flux. 3) A tripod pore water probe deploying passive samplers at different depth intervals both in the pore water and in the overlying water. The passive samplers are attached to a single steel probe penetrating the sediment bed to a predefined depth. The compact design of the probe ensures that the influence from the presence of the probe will have minimal effect on the overlaying water movements and therefore also minimal influence on the in situ sediment to water mass transfer coefficient and on the bioturbation activity of the benthic fauna. The novel tripod pore water probe is designed to be deployed at deep waters (used down to 120 m depth). The probe is also fitted with a releasable camera to monitor the deployment and to document the penetration depth of the probe in the sediment. This makes it possible to determine which passive samplers have been exposed to the pore water and which to the overlying waters. These new tools have been used to study the effect on sediment-to-water fluxes both from remedial actions such as capping and activated carbon amendment, and from natural mechanisms such as bioturbation and natural recovery processes in contaminated sediments.

481 Adapting passive samplers to investigate PAH and PCB flux from soil to air

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Magnitude and direction of flux of PCBs and PAHs were calculated using passive sampling devices to measure both air and soil pore air, that is, air that is in close proximity and equilibrium with soil. Existing methods to measure flux of semi-volatile compounds between soil and air require collecting soil samples from the top soil layer. This surface layer is hard to define, and existing extraction methods may overestimate the fraction of compounds available for partitioning into air in situ. In this modified design for sampling soil pore air in situ, an air-tight box is placed over low-density polyethylene passive samplers deployed 1 cm above the soil. Air exchange is limited to air that has equilibrated as it permeates through soil. In addition, passive air samplers were co-deployed at a height of 1.5 m above the soil to measure concentrations in air. Sampling occurred adjacent to two Superfund sites: a former PCB manufacturing facility in Anniston, Alabama, and a former creosoting site near Seattle, Washington. Following extraction in n-hexane, samplers were analyzed for PCBs with dual gas chromatography-electron capture detectors, and for PAHs with gas chromatography-mass spectrometry. Air and pore air concentrations were calculated using performance reference compounds. Flux calculations followed Fick's law of diffusion. Higher molecular-weight compounds were generally found to be volatilizing from the soil, while lower molecular weight compounds were depositing into the soil. Inter-sampler variability was greater among pore air samplers than air samplers, likely due to soil heterogeneity. This adapted method of measuring pore air in situ will allow for increased understanding of source-sink dynamics at sites with historical contamination.

482 Benthic Injury Dose-Response Models for PCB-Contaminated Sediment Using Equilibrium Partitioning

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The goal of this study was to develop a sediment PCB dose-response model based on benthic invertebrate effects to PCBs. We used an equilibrium partitioning (EqP) approach to generate predicted PCB sediment effect concentrations (largely Aroclor 1254) associated with a gradient of toxic effects in benthic organisms from effects observed in the aquatic toxicity studies. This report differs from all other EqP collective sediment investigations in that we examined a common gradient of effects rather than a single, protective value. We reviewed the chronic aquatic toxicity literature to identify measured aqueous PCB concentrations and the associated benthic invertebrate effects. We control-normalized the aquatic toxic effect data and expressed results from various studies as a common metric, % injury. Then we calculated organic carbon (oc)-normalized sediment PCB concentrations (mg/kg-oc) from the aqueous PCB toxicity dataset using EqP theory based on EPA's (EPIWEB 4.1) derivation of Koc. Lastly, we constructed a non-linear dose-response numerical model for these synoptic sediment PCB concentrations and biological effects (Y = 100/1 + 10(logEC50-logX) • (Hill slope)). These models were used to generate easy to use "look-up" tables reporting % injury in benthic biota for a range of Aroclor-specific sediment concentrations. For example, the model using the EPIWEB 4.1 Koc estimate predicts the mean benthic injury of 23.3%, 46.0%, 70.6%, 87.1% and 95% for hypothetical sediment concentrations of 1, 2, 4, 8, 16 mg/kg dry wt. of Aroclor 1254, respectively (using 1% oc). Models for some Aroclors (1016, 1221, 1232, and 1268) could not be developed due to data gaps toxicity literature. Specific step-wise procedures are provided for predicting % benthic injury when sediment PCBs are reported as Aroclor, congeners, homolog groups or total PCBs. The report identifies and discusses the uncertainties associated with the numerical PCB dose-response models and the EqP approach and provides considerations for how other Koc values result in more or less conservative models. This paper provides recommendations for addressing outstanding issues, including the Koc calculation, the two-carbon model, and congener data. We recommend using the model presented for screening but suggest, when possible, to determine a site specific Koc; that along with the tables and equations herein allows users to create their own protective dose-response sediment concentration for a specific locale.

483 Comparison of modeled and measured persistent organic pollutant flux between sediments and water column on the Palos Verdes Shelf

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The Palos Verdes Shelf (PVS) Superfund site is in over 50 meters of water on the continental shelf and slope off the coast of southern California (USA). The site includes more than 25 km^2 of sediments contaminated over several decades by municipal treatment plant effluent discharged via off-shore outfall pipes. Contaminants of concern include polychlorinated biphenyls (PCBs) and the pesticide DDT and its degradation products (e.g., DDE). Understanding the rate of exchange of these compounds between environmental media including sediments, water column and organisms, and the rate of transport off of the shelf are critical to predicting the fate of the persistent organic pollutant (POP) inventory at the site. In this work, results from water column and sediment porewater monitoring campaigns that employed passive sampling methods were used to calculate the flux of POPs between sediments and the water column and the advective transport of dissolved contaminants along the shelf. Further, the results were used as inputs to multi-box models of the Palos Verdes Shelf water column. The simplest model, a series of boxes along the 60-m isobath, using calculated molecular diffusive flux from the sediment bed to the water column as the only POP input, matched observed water concentrations within 70% for DDE and PCB congener 52. This supports the assumption that molecular diffusive flux between the sediment and water column media is a principal source of POPs to the water column at this site. In addition, box models were used to determine the potential magnitude of unaccounted sources or sinks for POPs on the shelf, and the time required to reduce sediment concentrations to values targeted by remedial project managers assuming only diffusive and advective transport of dissolved compounds.

484 Diffusive Flux of PAHs Across Sediment, Water, and Air Interfaces at Urban Superfund Sites

J. Minick, K.A. Anderson, Oregon State Univ / Environmental and Molecular Toxicology

Urban centers are often sources of polycyclic aromatic hydrocarbons (PAHs) to the surrounding environment. Knowledge of PAH transport and fate is important for understanding their relative source contributions which can help to inform remedial and management decisions. In this study, low density polyethylene (LDPE) passive samplers were co-deployed in sediment, water, and air, along the Willamette River in the city of Portland, Oregon. Sampling was conducted within and outside of the Portland Harbor Superfund Megasite (PHSM) and the McCormick and Baxter (MCB) Superfund Site. These Superfund sites have historical PAH contamination from industrial activities as well as ongoing PAH emissions from the Portland urban center. Samplers were analyzed for 62 PAHs using GC-MS/MS. Performance reference compounds were used to calculate freely dissolved PAH concentrations in water and sediment porewater and vapor phase concentrations in air. Flux due to diffusion was calculated across interfaces of the all three compartments following Fick's first law of diffusion. Results indicate that two and three-ring PAHs moved primarily from the sediment and the air into the water. This suggests that the Willamette River acts predominately as a sink for lighter molecular weight PAHs from both the sediment and the air. Additionally, comparison of these flux magnitudes suggest a similar level of source contribution. Sediment was also a source of four and five ring PAHs to the water at most locations. However, in contrast to the lighter molecular weight PAHs, the water was a source of these higher molecular weight PAHs to the air. At the remediated MCB Superfund Site, flux patterns reveal locations where contaminants may be breaking through the sand and rock sediment cap.

485 Distribution and Air-Water Exchange of Organic Flame Retardants in the Lower Great Lakes

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Organic flame retardants (OFRs), including polybrominated diphenyl ethers (PBDEs) and novel halogenated flame retardants (NHFRs), are ubiquitous, persistent contaminants that have been liberally added to a wide variety of consumer goods to slow combustion in the event of a fire. Recent studies have measured concentrations of OFRs in air, rain, and surface waters of the lower Great Lakes, but there are no published studies of regional spatial trends, nor are there reported measurements of air-water exchange fluxes. Polyethylene passive samplers (PEs) were deployed throughout the lower Great Lakes (Lake Erie and Lake Ontario) from 2011 to 2014 to (i) determine baseline concentrations of OFRs in air and water at a variety of shoreline, nearshore, and offshore sites, (ii) calculate exchange fluxes to determine whether the lakes were acting as sinks or secondary sources via diffusive air-water exchange, and (iii) investigate spatial trends and correlation with human population density. Dissolved Σ_{12}BDE was greatest in Lake Ontario near Toronto (18 pg/L) and gaseous Σ_{12}BDE was greatest on the southern shoreline of Lake Erie (11 pg/m^3). Air-water exchange was dominated by absorption of BDEs 47 and 99, ranging from -964 pg/m^2/day to -30 pg/m^2/day. Correlation between population and dissolved ?$_{12}$BDE was strongest when using a radius of 25 km around each site, while correlation with gaseous Σ_{12}BDE was strongest when using local (within 3 km) population to the south of each site, possibly due to the prevalence of southerly winds during the sampling campaign. Results for OFRs will be compared to other compound groups with distinct sources and usage histories, including polychlorinated biphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs), which were measured in the same extracts. Bayesian kriging will be utilized as a geostatistical interpolation technique to predict dissolved OFR concentrations over the lakes, illustrating the utility of relatively highly spatially resolved measurements in identifying potential hot spots for future study.

486 Air-water exchange of POPs across the Atlantic Ocean

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The research cruise KN210-04 of the R/V Knorr in March-May 2013 from Uruguay to Barbados USA offered a unique opportunity to determine gradients of persistent organic pollutants (POPs) in air, water and their air-water exchange. The cruise track covered a fairly large north-south gradient off the South-American coast, along Brazil into the Caribbean, so far unexplored in terms of POPs concentrations and fluxes, far away from shore. Concentrations of PCBs and OCPs in the air ranged from < detection limit to 15 pg/m3, dominated by PCB 28 and hexachlorobenzene (HCB). In the water, concentrations were typically < 5 pg/L, with HCB and PCB 52 as the major compounds. Of the polybrominated diphenyl ethers (PBDEs), mostly only congeners 47 and 99 were detected in the gas-phase, at up tp 10 pg/m3, while dissolved concentrations were mostly at around 2 pg/L. Detection in water samples were spotty, implying larger-scale currents responsible for the presence and absence of measurable POPs concentrations. Air-water exchange gradients for PBDEs implied strong net gas-phase deposition along the western Atlantic Ocean.

487 A Rapid Decline of Persistent Organic Pollutant Concentrations in the Sarasota Bay, FL Bottlenose Dolphin Population

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Declining trends of legacy persistent organic pollutants (POPs) have most frequently been documented in food webs from arctic and subarctic environments. Because of cold temperatures and slow degradation rates, arctic environments are considered sinks for POPs; however decay rates may not be reflective of other areas. Conversely, in tropical or subtropical environments, environmental conditions likely favor more rapid declines in POP concentrations with time because of warmer temperatures leading to greater POP volatilization and degradation. We hypothesized that POPs will decline with time more rapidly in a subtropical food web than samples from temperate or arctic food webs. Samples from the long-term resident Sarasota Bay bottlenose dolphin population provide an excellent opportunity to investigate POP trends in a subtropical estuary. The majority of bottlenose dolphins inhabiting Sarasota Bay are resident and have been cataloged through photo identification and nearly annual capture-release studies. Capture-release studies also provide access to blubber, blood and milk for POP analysis. Full depth blubber biopsies were collected from 2000 to 2016 (n=282) and analyzed for a suite of POPs including polychlorinated biphenyl congeners, organochlorine pesticides and polybrominated diphenyl ethers. Results for samples analyzed from 2000 to 2012 show large and significant declines of POPs in blubber. For example median wet weight blubber concentrations in males sampled from 2000 (n=12) to 2012 (n=8) declined from 26 mg/kg wet to 2.8 mg/kg, from 3.2 mg/kg to 0.32 mg/kg and from 0.91 mg/kg to 0.23 mg/kg for PCB 153, mirex and PBDE 47, respectively. Concentration declines were on the order of 20% per year for samples collected from 2000 to 2012. A meta-analysis of POP trends occurring in more northerly environments confirms that POPs in Sarasota dolphin blubber are declining at a faster rate than observed elsewhere. Concentration declines may contribute to improving health of Sarasota dolphins discounting other factors.

EDCs and Pharmaceuticals in the Environment – Part 2

488 Altered antimicrobial susceptibility in stream bacterial isolates exposed to triclosan

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Globally, the development and transmission of multidrug-resistant (MDR) bacteria is a major medical challenge. A commonly used antimicrobial, triclosan, is detectable in human and environmental samples. Triclosan is associated with numerous health risks including increasing antimicrobial resistance in bacteria. Environmental exposures to triclosan can alter susceptibilities of bacteria and studies have shown development of resistance to triclosan and cross-resistance to antibiotics in clinical isolates. Bacteria can obtain antimicrobial resistance factors through horizontal gene transfer. If acquired resistance and cross-resistance in environmental bacteria is developing in streams or wastewater (WW) treatment plants, this poses a human health risk. The overall goal of the current study is to evaluate stream bacterial responses to environmentally-relevant triclosan exposure. Antimicrobial susceptibility was evaluated in periphyton bacteria from a WW-associated stream and forested, reference stream. Reference bacteria were hypothesized to exhibit greater susceptibility to triclosan than WW-associated bacteria. Exposure to triclosan was hypothesized to affect susceptibility. Reference bacteria were expected to experience a greater effect of exposure on susceptibility compared to WW-associated. Resistance to triclosan was hypothesized to associate with resistance to other antibiotics. Periphyton was exposed to 10 ppb triclosan in microcosms, then random isolates were cultured. Unexposed and exposed isolates from each stream were evaluated for susceptibilities to triclosan and five antibiotics through broth microdilution assays. Among unexposed isolates, higher overall susceptibility was observed in reference stream isolates compared to WW-associated isolates. Exposed WW-associated isolates show similar triclosan susceptibility the unexposed WW-associated isolates. Exposed reference stream isolates show lower triclosan susceptibility when compared to unexposed reference isolates. Similar trends were also observed with some of the other antibiotics tested. Most isolates showing resistance to the doses of triclosan tested also show low to no susceptibility to carbenicillin, chloramphenicol, erythromycin, and trimethoprim. Results of this study indicate that low levels of triclosan in streams may alter susceptibilities of environmental bacteria to triclosan and other antibiotics, possibly adding to the challenge of development of MDR bacteria.

489 Spatio-temporal bioaccumulation and trophic transfer of ionizable pharmaceuticals in a semi-arid stream influenced by snowmelt

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Bioaccumulation of pharmaceuticals and personal care products (PPCPs), and other contaminants of emerging concern (CECs) in aquatic systems has increasingly been reported in the literature. It is critically important to recognize that traditional approaches to understand and predict exposure, accumulation, and effects of classic contaminants (e.g., PCBs) may not be appropriate for many CECs. For example, many ionizable pharmaceuticals typically have lower log P values, are more water soluble, and accumulation is influenced by pH. However, during dry periods and droughts, base flows in rivers are increasingly dominated by or dependent on effluent discharge, resulting in longer exposure durations to aquatic life. Such urban waters represent worst case scenarios for potential aquatic exposure and ecological effects from consumer products. Exposure and accumulation of pharmaceuticals can be influenced by different species specific fitness traits (foraging range, feeding behavior, migration, morphology) and differential ability among species to biotransform compounds. Because a comprehensive review of pharmaceuticals and aquatic organisms recently identified that studies examining spatial and temporal factors influencing exposure, bioaccumulation, and risk of pharmaceuticals to aquatic life is decidedly lacking, we examined the spatio-temporal bioaccumulation of select pharmaceuticals in multiple trophic positions (periphyton, invertebrates, and fish) from East Canyon Creek, Park City, Utah, at increasing distances downstream from an effluent discharge (0.15, 1.44, and 13 miles) during three seasons (spring, summer, and fall) of 2014. Stable isotopes analysis identified trophic positions within functional food chains, and spatio-temporal trophic magnification factors (TMFs) were determined. Results of the current study emphasize the significance of characterizing pharmaceutical bioaccumulation in relation to seasonal and longitudinal variations, in pharmaceutical occurrence, and exposure scenarios based on species behavior and habitat use. Such considerations appear important for future bioaccumulation assessments of ionizable contaminants.

490 Stress-related symptom and detoxification mechanisms induced by PPCPs in plants

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Crops irrigated with reclaimed wastewater may take up and accumulate pharmaceutical and personal care products (PPCPs), which have been recently shown to affect plant physiology and alter normal biochemical pathways. Existing studies tested toxicity of individual compounds. However, effluent-irrigated crops are exposed to PPCPs as mixtures, understanding mixture toxicity may be important for predicting plant bioaccumulation of PPCPs. The present study explores the uptake, translocation and oxidative stress response induced by 18 frequently-occurring PPCPs (100 $\mu g\ L^{-1}$) in cucumber seedlings grown in nutrient solution for 20 days. PPCPs concentration in leaves and roots is determined by HPLC-MS, and bioconcentration factor and translocation factor are calculated. Oxidative stress parameters, such as lipid peroxidation, cell death, hydrogen peroxide and superoxide content are determined both in roots and leaves. PPCPs induced root cell death, especially in root tips. MDA content was also enhanced by PPCPs. Non-antioxidant enzymes (ascorbic acid, glutathione) and antioxidant enzymes (superoxide dismutase, catalase, glutathione reductase and peroxidase) are evaluated. These results provide phytotoxic effects induced by PPCPs mixture, strengthing the need for the evaluation of the ecotoxicological effects of wastewater treated flows.

491 Bioaccumulation of pharmaceuticals and other emerging contaminants: What's in the lower aquatic trophic levels?

J. Wilkinson, P.S. Hooda, Kingston Univ London / Natural and Built Environments; J. Swinden, J. Barker, S. Barton, Kingston Univ London / Life Sciences Pharmacy Chemistry

Organic contaminants such as pharmaceuticals, personal care products (PPCPs) and other emerging contaminants (ECs) are known to persist in the aquatic environment and many are indicated as endocrine, epigenetic, or other ecotoxins. Typically, the study of such compounds in the aquatic environment is limited to their occurrence dissolved in river water. In this study, accumulation of thirteen PPCPs/ECs was assessed in aquatic sediment (n=23), plants Callitriche sp. (n=8) and Potamogeton sp. (n=7) as well as amphipod crustacean (Gammarus pulex, n=8) and aquatic snails (Bithynia tentaculata, n=6). All samples were collected from the Hogsmill, Blackwater and Bourne Rivers in southern England. The Hogsmill and Blackwater Rivers were studied from headwaters to the first confluence with another river. Targeted PPCPs/ECs included pharmaceuticals acetaminophen, diclofenac, and ethinylestradiol, plasticisers bisphenol-A (BPA), bisphenol-S (BPS) and 4'-hydroxyacetophenone (HAP), perfluorinated compounds (PFCs) perfluorooctanoic acid (PFOA), perfluorooctanesulfonic acid (PFOS), perfluorononanoic acid (PFNA) and perfluorobutane sulfonic acid (PFBS), and illicit drugs/metabolites methamphetamine, amphetamine and benzoylecgonine. Contaminants were extracted using ultrasonic-assisted extraction and analysed using an in-house validated method for solid phase extraction followed by liquid-chromatography tandem mass spectrometry. Ten of thirteen analytes were detected in sediments with frequencies ranging from 22-83% (benzoylecgonine and BPA respectively) and mean quantifiable concentrations ranging from 0.84-11.1 ng/g dry weight (dw) (BPS and BPA respectively). Seven of thirteen analytes were detected in Callitriche sp. with frequencies ranging from 13% (acetaminophen) to 100% (HAP, BPS, PFBS and PFOA) and mean concentrations ranging from 0.42-113 ng/g dw (diclofenac and PFOS respectively). Eight of thirteen analytes were detected in Potamogeton sp. with frequencies ranging from 14-100% (acetaminophen and HAP respectively) and mean concentrations of 0.38-71.0 ng/g dw (acetaminophen and HAP). Lower levels of selected contaminants were found in aquatic snails (0.69-25.8 ng/g dw, acetaminophen and PFBS respectively) and amphipod crustaceans (0.32-14.2 ng/g dw, BPA and PFNA respectively). These data form a detailed accounting of PPCP/EC fate and distribution in the aquatic environment highlighting accumulation at bottom trophic levels, a potential source for higher organisms.

492 Occurrence and Estrogenic Burden of Five Parabens in Sewage Sludge from the United States

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Parabens are a group of compounds that have been extensively used as preservatives in pharmaceutical and personal care products (PPCPs), food, beverages, and industrial products. Continuous use and discharge of parabens into the environment can lead to occurrence at steady-state levels despite their susceptibility to degradation. As parabens are considered to represent emerging endocrine disruptors and have raised concerns for human and animal health, monitoring the occurrence of parabens in the environment is important for both ecological and human health risk assessments. In this study, five parabens were quantified by liquid chromatography-tandem mass spectrometry (LC-MS/MS) in sewage sludges collected at 14 U.S. wastewater treatment plants (WWTPs) located in nine states. Detected concentration ranges (in units of ng/g dry weight sludge) and frequencies were as follows: methyl paraben (15.9 to 203.0; 100%), propyl paraben (0.5 to 7.7; 100%), ethyl paraben (< 0.6 to 2.6; 63%), butyl paraben (< 0.4 to 4.3; 42%) and benzyl paraben (< 0.4 to 3.3; 26%). The estrogenicity inherent to the sum of parabens detected in sewage sludge (ranging from 10.1 to 500.1 pg/kg in units of 17β-estradiol equivalents) was insignificant when compared to the 10^6-times higher value for natural estrogens, calculated based on literature data for sewage sludge. This is the first report on the occurrence of five parabens in U.S. sewage sludges. Study results will help to inform the risk assessment of biosolids, i.e., treated sewage sludge destined for application on land.

493 Risks Associated with the Environmental Release of 15 Active Pharmaceutical Ingredients on US Food and Drug Administration's "Flush List"

U. Khan, FDA/CDER / Center for Drug Evaluation and Research; J.P. Laurenson, R.A. Bloom, USFDA / Center for Drug Evaluation and Research

A select few prescription drugs can be especially harmful and, in some cases, fatal with just one dose when not used as prescribed. To prevent accidental ingestion of these drugs by children, or pets, the US Food and Drug Administration (FDA) recommends that unused portions of these drugs be disposed quickly through a take-back program. If such an option is not readily available, FDA recommends that they be flushed down the sink or toilet. The goal of the current investigation was to evaluate the sources and risks associated with the environmental release of the 15 active pharmaceutical ingredients (APIs) currently on this so-called "flush list". This list consists of 10 opioids – buprenorphine, fentanyl, hydrocodone, hydromorphone, meperidine, methadone, morphine, oxycodone, oxymorphone, tapentadol; 1 benzodiazepine – diazepam; 1 psychoanaleptic – methylphenidate; 1 nervous system depressant – sodium oxybate; and 2 opioid antagonists – naloxone, naltrexone. Source models were developed for the release of these APIs to US waterways. For some APIs, the models revealed the importance of sources other than their clinical use (e.g., endogenous production and excretion, the use of illicit drugs). A risk-based evaluation was performed to assess the environmental and human health relevance of the flushed APIs. Exposure assessment was performed using both modelled and, where available, measured concentrations. Eco-toxicological effects assessment was conducted by gathering eco-toxicological data from various sources. For those compounds for which sufficient eco-toxicological effects data was not available, the fish plasma model was used to establish the need for additional fish chronic studies. Safe levels for human-health exposure through drinking water were estimated by extrapolating the lowest oral therapeutic doses to no-effect concentrations. To date, the evaluation suggests that most of these APIs clearly present a de-minimis environmental risk, while for some, fish chronic endpoints will need to be developed. All APIs present de minimis human health risk through drinking water. Several caveats exist and additional data are needed to: (1) fully understand the significance of manufacturing as a contributor to environmental loading of such chemicals, (2) evaluate the potential to form hazardous transformation products, and (3) address mixture effects. The contents of this abstract neither constitute nor reflect official US FDA policy.

494 Role of Effluent Organic Matter in the Photodegradation of Compounds of Wastewater Origin

A. MacKay, The Ohio State Univ / Civil Environmental and Geodetic Engineering

Treated municipal effluent discharges are an important source of compounds that may be biologically active in aquatic systems. The co-released effluent organic matter from the engineered microbial treatment process may influence compound degradation by decreasing the available sunlight for direct photodegradation, or increasing production of triplet excited state organic matter (^{3}DOM*), singlet oxygen ($^1O_2$) or hydroxyl radicals (OH-dot) for indirect photodegradation. Degradation rates for common wastewater effluent compounds – sulfamethoxazole (direct photodegradation), sulfadimethoxine (^{3}DOM reaction), cimetidine ($^1O_2$ reaction) and caffeine (OH-dot reaction) – were measured in upstream river water, treated effluent and mixtures thereof. A conservative mixing model for estimating compound degradation rates from river water and effluent end members could predict degradation rates in mixtures for direct photodegradation and OH-dot reactions. Compound degradation reactions via ^{3}DOM* and $^1O_2$ exhibited quenching in mixtures such that observed reaction rates were lower than estimated. The high photoreactivity of effluent organic matter and its susceptibility to quenching by river organic matter seems to be consistent with effluent organic matter phenol content. Overall, compound degradation rates were correlated with the simple optical parameter, E_2/E_3 ratio (sample absorbance at 254 nm divided by absorbance at 365 nm), for all water samples and mixtures. This finding opens the door to predictive estimations of compound attenuation capacity via photodegradation in waters downstream from treated wastewater effluent discharges.

495 Optimizing Operating Parameters to Enhance the Removal of Emerging Contaminants in Wastewater Treatment Plants Using the STP Model

A. Benedicta, Univ of Houston Clear Lake / Biological and Environmental Science; C. Zhang, Univ of Houston Clear Lake / Dept of Environmental Sciences

Wastewater treatment plants (WWTPs) have become a significant source of emerging contaminants of concern; however, conventional WWTPs are not effective for the removal of emerging contaminants such as metabolites of alkylphenol ethoxylate surfactants, steroid hormones, plasticizers, pharmaceuticals and personal care products. The aim of this study was to evaluate if the removal efficiency can be improved by optimizing operational parameters through the simulation in a fugacity based sewage treatment plant (STP) model. Seven commonly detected organic wastewater contaminants in the U.S. streams, i.e., pyrene, bisphenol A (BPA), estriol, triclosan, diazinon, 4-nonylphenol and tetrachloroethylene (PCE) were subjected to STP modelling by varying hydraulic retention time (HRT), solids retention time (SRT), mixed liquid suspended solids (MLSS), and aeration rate. Results showed that the total % removal for all contaminants increased with increasing HRTs, particularly for BPA and estriol with relatively low logKow pertaining to their low sorption constants. Under optimized HRTs, % improvement for BPA and estriol removal was significant (ranged approximately 8~35% and 9~47%, respectively) and moderate for diazinon and PCE. Total removal for all contaminants increased with SRT, and the % improvement with increasing SRT was moderate for BPA and estriol, and slight for pyrene, triclosan, diazinon, 4-nonylphenol and PCE. Simulations with increasing MLSS failed to improve total removal for all contaminants and the optimal removal was achieved at 1000 mg/l which is markedly lower than the operating MLSS currently in practice for conventional plug flow and CFSTR activated sludge systems. With an increasing aeration rate, the result showed that the total % removal, % biodegradation removal, % sludge removal and % volatilization removal was the same for all contaminants except for PCE, indicating the trade-off between the removal and fugitive emissions for highly volatile compounds. While STP has its inherent limitations for the process modeling in WWTPs, our overall attempt clearly indicates the potential of improved treatment efficiency by optimizing operational parameters currently in use. This work also points to the fact that maintaining an extended HRT in some WWTPs is subjective to further feasibility evaluation, and the strategy of increasing SRT for an improved treatment efficiency need to be examined on each individual contaminant basis.

Making Your Research Relevant to Regulatory Science and Supportive of Decision Making

496 It's a two-way street... or at least it should be

V. Forbes, Univ of Minnesota / Ecology Evolution Behavior

Ensuring that our science is relevant to decision making and that our decision making is informed by good science should be an iterative process with multiple feedbacks in both directions. It should be a process that continuously improves as scientific knowledge, technological developments, and lessons learned from previous decisions all evolve. Unfortunately, studies conducted to address specific research questions do not necessarily meet the rigorous standards upon which regulatory risk assessments should be based. Yet, there can be hesitance on the part of regulators to discard or downplay such studies, particularly if their findings suggest that risks are high. On the other hand, when scientific evidence argues for improving the design and/or reporting of regulatory test protocols, there can be tremendous inertia in getting the needed improvements implemented in revised test protocols. This presentation will provide an example of each of these (the former related to the risk assessment of bisphenol-A in Europe, and the latter related to changes in the design of chronic reproduction test protocols. A conclusion is that both science and regulatory decision making could benefit from more seamless and efficient feedback.

497 Research Supportive of Aquatic Life Ambient Water Quality Criteria Development

K. Gallagher, M. Elias, J.R. Beaman, D.M. Eignor, USEPA / Office of Water

The U.S. Environmental Protection Agency's Office for Water develops Aquatic Life Ambient Water Quality Criteria recommendations (also known as 304(a) criteria) using available toxicity test data, typically data available through the open literature. The Clean Water Act, the statute under which Water Quality Criteria are developed and applied, does not enable EPA to directly request or require data generation by chemical manufacturers, unlike pesticide and industrial chemical statutes in the US. Therefore, the need for high quality, reproducible research is heightened for the development of robust Aquatic Life Criteria. EPA occasionally needs to contact publication authors in order to access underlying data, such that more complex statistical analyses can made, in addition to typical metrics reported in the open literature (e.g., LC_{50}, EC_x). Thus, ensuring data are gathered and reported in a way that facilitates further analysis would increase the utility of academic research for criteria development. Additionally, conducting research that supports development of quantitative linkages between sublethal effects and effects of regulatory concern, typically survival, growth and reproduction, would be useful to government regulators. For example, reporting results indicating binding of a particular receptor following chemical exposure, or effects on olfaction, would be most useful when tied quantitatively to an apical endpoint. Enhanced application of research can be furthered by increased information sharing and contact between academic and regulatory scientists through forums such as SETAC.

498 Making your research relevant to ecological risk assessments conducted by Environment and Climate Change Canada

J. Hill, A. Gosselin, D. Gutzman, Environment and Climate Change Canada / Ecological Assessment Division; N. Khera, Environment and Climate Change Canada / Emerging Priorities Division

Under the Canadian Environmental Protection Act, 1999, Environment and Climate Change Canada (ECCC) assesses and manages, where appropriate, the ecological risks of substances. When conducting risk assessments, ECCC gathers and evaluates studies obtained from a variety of sources, including peer-reviewed journals, environmental databases and industry submissions. Studies are useful to document physical and chemical properties of chemicals, their environmental fate and behavior (including persistence and bioaccumulation) as well as their toxicological effects on organisms. These studies also occasionally provide information on environmental concentrations of chemicals in water, sediment or soil. Environmental databases (e.g., from provincial and territorial governments in Canada) can be an invaluable source of data on environmental concentrations of chemicals. For ECCC to be able to fully evaluate and use physical-chemical, ecotoxicological and exposure data from studies and environmental databases, certain key ancillary information regarding study conditions must be available and clearly communicated in the published studies or in databases (or in readily available supporting documentation). Also, as ECCC and other regulatory agencies in the world are assessing an increasing number of chemicals for which very little data are available to conduct a risk assessment, the results of studies conducted on these chemicals can be highly critical to support regulatory decision-making. For such results to be fully useful, they ideally need to be aligned with priorities identified for risk assessments by regulatory agencies in the medium and long term (e.g., ≥3 years). This presentation will provide examples of how certain research results from peer-reviewed articles, industry submissions and environmental databases have been used in the past in risk assessments conducted by ECCC, and how they have supported regulatory decisions. Examples of research results, including their evaluation through use of Robust Study Summary forms, that could not be used, or that were used with caution, in risk assessments due to a lack of reported information on study protocol or results obtained will also be presented.

499 Better designing and reporting of animal studies to support regulatory decision making

N. Burden, NC3Rs

Regulatory bodies are increasingly considering data from the open literature when making decisions on chemical safety. Uncertainty regarding results reported in the literature can trigger new ecotoxicity studies for regulatory purposes. It is therefore crucial that published data is of the highest quality possible. This will ensure that all of the available information can be utilised, and that additional studies are only conducted where necessary. There is a particular need for in vivo studies to be robustly designed so that the information gained is reliable and repetition of studies minimised, reducing the overall numbers of animals that are used to meet the scientific objectives. Adherence to reporting guidelines such as ARRIVE (Animal Research: Reporting of In Vivo Experiments) also plays a central role in supporting the interpretation of published data, by ensuring that studies are comprehensively reported in a transparent manner. A programme led by the UK's National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs) continues to address the key issues related to experimental design, analysis and reporting. While relevant to all areas of research, the application of best practice in study design and reporting of research has obvious benefits to the field of ecotoxicology, especially considering the implications of such studies on regulatory decision making. The NC3Rs also leads an expert Working Group in regulatory ecotoxicology, comprising of scientists across academia, regulatory bodies and industry. This presentation outlines the activities we are undertaking to maximise the utility of published data across the biosciences, plus more specific priorities recently identified by the Working Group pertinent to using published data in regulatory decision making.

500 Designing and Conducting Chemical Fate Research to Support Risk Assessment

M.T. Lee, USEPA / Office of Pollution Prevention and Toxics; K. Eisenreich, C.D. Penalva-Arana, USEPA / OPPTRAD

EPA's Office of Pollution Prevention and Toxics (OPPT) is responsible for evaluating new and existing chemicals for human health and ecological risk, under the Toxic Substances Control Act. Chemical fate in the environment is one aspect of risk assessment that informs regulatory decisions. Fate assessment incorporates data generated by academia, research laboratories, and industry. This talk will provide a background on the environmental fate "endpoints" used by EPA's OPPT fate assessors, will address common reporting errors and emissions made when reporting data generated using test guidelines, highlight specific deficiencies in chemical fate data availability, and suggest key contributions the research science community could make towards improving fate assessment that supports decision making. For example, interpretation of chemical fate in the environment is hindered by a lack of data on chemical degradants and metabolites in environmental systems. Further development in this research area has the potential to greatly advance risk assessment science. The views of the authors of this abstract are those of the authors and do not represent Agency policy or endorsement.

501 The use of environmental exposure data to support regulatory risk assessments for octamethylcyclotetrasiloxane (D4)

K. Thomas, Global Silicone Council

The availability of empirical environmental exposure data to support regulatory evaluations for industrial chemicals is often limited. This scarcity of exposure data is often made more complicated because the environmental fate models that are frequently used to estimate environmental exposure are not well suited for predicting the environmental loadings associated with many chemicals. Compounds that don't behave similarly to the carbon-based chemicals which have historically been used as training sets for development of environmental exposure models are especially challenging for predicting environmental exposure. D_4 is a compound where environmental exposure estimates are difficult using classical environmental exposure models. Consequently, generating empirical environmental exposure data is essential for developing scientifically-defensible regulatory environmental safety evaluations for D_4. Although environmental exposure data for D_4 has been generated by scientists in government, academia, and industry, these data have not been consistently used to support regulatory evaluations for D_4 in North America and Europe. Assessment of the persistence (P), bioaccumulation (B), and toxicity (T) characteristics of industrial chemicals, which represents the core of a risk-based environmental assessment, is benefited considerably by the use of reliable and robust environmental exposure data. The presentation will review the available environmental exposure data for D_4, highlight the extent to which environmental exposure data was used to support regulatory evaluations of D_4 in Canada and Europe, and review the impact on the outcome of those assessments from the use of environmental exposure data. The presentation will also explore the approaches used for the completed environmental assessments of D_4 conducted by Canada and Europe, and review the data that is being generated to support an environmental risk assessment of D_4 in the US.

502 Regulatory guided research to improve biodegradation assessments

T. Martin, Newcastle Univ / School of Civil Engineering and Geosciences; J. Snape, AstraZeneca UK Ltd. / AstraZeneca Global Environment; R. Davenport, Newcastle Univ / School of Civil Engineering and Geosciences

Microbial degradation is an important but poorly understood process that can determine the fate of organic chemicals in the environment. The investigation of biodegradation in the environment is stuck in the paradigm of low throughput "one sample-one test" systems that often ignore the considerable variation inherent in natural microbial communities. Systems such as these form the core basis for regulatory screening tests, including the OECD Ready Biodegradability Tests (RBTs) used for hazard classification, environmental risk assessment and persistency assessments. The Registration, Evaluation, Authorisation and restriction of CHemicals (REACH) was introduced in 2007 to streamline existing legislation. REACH advocates the prioritisation of chemicals based on hazards including persistence and acknowledges the limitations associated with existing screening tests, suggesting a series of enhancements and modifications to improve their reliability. RBTs are notoriously highly variable, producing a large number of false negatives which result in unneccessary, costly, higher tier assessments of chemicals which are inherently biodegradable. REACH requires Persistency, Bioaccumulation and Toxicity (PBT) data for all new and existing chemicals produced in volumes greater than 100 tonnes per annum. Previous legislation only applied to "new" chemicals introduced to market after 1981, accounting for less than 4% of all commercially available products. High-throughput biodegradation screening tests (HT-BSTs) have the potential to identfiy those chemicals which pose the greatest risk to the environment from the vast number awaiting registration and prioritise them for more intensive assessment. This presentation will discuss the investigation of enhancements to RBTs, recommended within REACH guidance, to improve reliability and enable a more effective prioritisation on persistence. Ongoing efforts to translate this research into policy and the outcome of engagement activities between regulators, industry and academia will be reported. Recommendations will be delivered on the best practice for validating new biodegradation tests. This presentation will also consider the potential use of HT-BSTs within the existing regulatory framework.

503 Assessment of Ecotoxicity Data for Regulatory Risk Assessment

J.W. Green, DuPont / Applied Statistics Group; T.A. Springer, Wildlife International, Ltd.

There is understandable interest in driving the statistical evaluation of ecotoxicity data performed for regulatory risk assessment towards regression based estimates of effects concentrations (ECx) and away from hypothesis testing, e.g. NOEC. However, no single statistical approach can be applied to every response from every study of every type of experiment in ecotoxicology. Careful assessment is needed to determine which statistical approach is relevant and useful for specific situation. Ecotoxicity data includes continuous responses such as weight, length, and yield; quantal responses such as seedling emergence, hatching, and mortality; count data such as number of offspring or some fish sexual characteristics; histopaathology severity scores; and time-to-event responses, such as time to reach some developomental stage or time to hatch or swim up. Methods based on idealized data that ignore the complexities of dealing with these diverse endpoints and the difficult situations often encountered in real-world testing are detrimental to regulatory risk assessment and are poor science. Limit tests have only 1 treatment grouip. Endocrine disruption tests often have only 3 test concentrations. The latter determine whether evidence of endocrine activity exists, give an idea of what concentrations have that potential, and permit at best simplistic regression models and ECx estimates with wide confidence intervals. Avian reproduction and multi-generation tests have many endpoints with divergent statistical properties. Animal welfare considerations speak against the addition of more treatment groups. Non-target plant and fish early life-stage studies have 5 or more treatment groups to allow regression models. Even so, in well designed and well performed studies, the data sometimes do not support a useful mathematical model but do provide sufficient information to make sound regulatory decisions. Examples illustrate this and decades of experience and simulation studies show such examples are not rare. Statistical approaches for risk assessment must include careful consideration of the realities of the data that arise in guideline studies. Failure to do so can mislead and do a disservice to the ecotoxicity community. An illustrative example will be provided that also serves to illustrate recommendations from a recent SETAC Pellston Workshop on ways to improve the science of ecotoxicology.

Bringing Probabilistic Risk Assessment into Criteria Development

504 Selecting Target Risk Levels When Deriving Criteria Using Probabilistic Methods

P.D. Anderson, Arcadis US, Inc.; M. Buonanduci, ARCADIS

Human health water quality criteria (HHWQC) are established using assumptions for a variety of parameters. Those include several necessary to estimate possible exposure, at least one to estimate potential toxicity, and another to establish the target risk. When using a deterministic approach to develop HHWQC only a single target risk is necessary (e.g., $1x10^{-6}$ or $1x10^{-5}$). When probabilistic methods are used, multiple target risk levels can be used. For example, a specific percentile of the exposed population (or other statistical representation of the exposed population such as the arithmetic mean) can be targeted at a $1x10^{-6}$ excess lifetime cancer risk and a different percentile at a $1x10^{-5}$ excess lifetime cancer risk level. This presentation will summarize and compare the various target risk levels that have been proposed by various regulatory agencies. Another advantage of a probabilistic approach is that the level of protection afforded different segments of the population is transparent compared to the more commonly used deterministic approach. Even though an HHWQC derived using a deterministic approach is based on a specific target risk level (e.g., an excess lifetime cancer risk of $1x10^{-6}$ or a hazard quotient of 1.0), because of a combination of several conservative assumptions, one does not know what fraction of the population is expected to experience the target risk. This presentation will compare the levels of protection afforded different segments of the population based on various deterministically derived criteria.

505 Challenges associated with the use of probabilistic methods to develop human health WQC

S. Replinger, N. Lewis, J. Toll, N. Judd, Windward Environmental, LLC

We used a probabilistic approach as part of work to develop revised human health water quality criteria (WQC) for the Idaho Department of Environmental Quality. The use of a probabilistic approach has known advantages (primarily the opportunity to understand and account for the effects of inherent uncertainty or variability in parameter values on WQC) and disadvantages (primarily the increased level of effort and time required for calculations and to communicate with the public on the process). A challenge that is often not immediately apparent is the importance of understanding how changes in the distributions and percentiles of the population that are targeted can have different impacts on WQC for different chemicals (e.g., those with low vs high bioaccumulation factors). Along these lines, it is useful to consider how these probabilistically-developed criteria compare with criteria developed using standard deterministic methods. Despite these challenges, a probabilistic approach allows information regarding the resulting criteria and associated risks to be displayed visually and in the context of the population, which provides for a more transparent presentation of the results. In this presentation we will talk about the challenges we encountered, how we dealt with them, and what advice we'd offer to others who are interested in using a probabilistic approach to develop human health WQC.

506 Generating a Distribution of Usual Fish Consumption Rates for Applying PRA to Human Health Criteria

D. Essig, Idaho DEQ / Water Quality

Applying probabilistic risk assessment (PRA) requires distributions to describe the various inputs. One key exposure input to calculating human health criteria is the rate of fish consumption in the population to be protected. Unfortunately most available fish consumption surveys, particularly local and regional surveys, report rates for only selected points in a distribution, typically focusing on upper percentiles, e.g. 90th, 95th or 99th, ignoring the bulk of the distribution. This is insufficient for performing PRA. In addition, most available surveys report short-term fish consumption rates that are not representative of long-term AKA usual fish consumption rates. Chronic human health criteria should be based on long-term fish consumption behavior. Fish consumption rate surveys that develop the information necessary to estimate long-term fish consumption rates require unique survey methodology characteristics that mesh with processing methods, such as the National Cancer Institute method, designed to estimate long-term averages from short-term recall data. The presentation will describe the effort that was undertaken by Idaho to conduct a State-specific fish consumption survey and process the data resulting in a full distribution of long-term fish consumption rates appropriate for a PRA.

507 Evaluation of Fish Consumption Rates for use in Florida's PRA-based Human Health Criteria Derivation

K. Weaver, Florida Dept of Environmental Protection / Water Quality Standards

The fish consumption rate (FCR) in the target population or populations is a key exposure input to the calculation of human health criteria (HHC). In 2014, USEPA published FCRs for the U.S. population and selected subpopulations based on NHANES survey data for the period 2003 to 2010. Most relevant to the task of deriving HHC for Florida, EPA included trophic level 2, 3, and 4 FCRs for the Gulf Coast, Atlantic Coast, and Inland South subpopulation. Florida DEP used the three sets of FCRs to derive HHC for Florida based on a probabilistic risk analysis (PRA). Lognormal distributions were used to define all nine FCRs for use in the Florida PRA. These nine FCRs were used in conjunction with a custom distribution to generate Florida-specific FCRs. The custom distribution described the probability of an individual being a member of a given subpopulation, and was based on the proportion of the Florida population residing within each region. States and authorized tribes could potentially use a similar methodology, regardless of whether a probabilistic or deterministic approach is used, to generate more state-specific FCRs for purposes of their own HHC calculations as an alternative to the national default rates. Additionally, Florida DEP also investigated, through a sensitivity analysis, the effect of assuming correlations between fish consumption and body weight. It was determined that assuming no correlation resulted in the broadest distribution of population risks from the PRA. Increasing the assumed correlation between BW and FCR resulted in a narrower risk range, decreased the average risk, decreased the 90th percentile risk, and ultimately would increase the HHC value.

508 Relative Source Contribution: Capturing the Full Potential of Exposure Characterization in Criteria Development

K. Summerfield, Florida Dept of Environmental Protection

States are tasked with developing surface water quality standards that are protective of human health with regard to ingestion of aquatic organisms and surface water derived drinking water. One input variable important to the derivation of human health criteria for non-carcinogenic parameters is the relative source contribution (RSC), which is a quantitative input that accounts for exposures from sources other than water and fresh/estuarine fish and shellfish ingestion (i.e., inhalation, other dietary exposures, soil and dust ingestion). The RSC can be calculated through two methodologies, the percentage approach and the subtraction approach, both of which are constrained by a floor of 0.20 and a ceiling of 0.80 as defined by the USEPA's policy. The Florida Department of Environmental Protection (department) attempted to develop parameter-specific RSCs alternative to the default floor and ceiling values used by the USEPA as part of their 2015 HHC recommendations. For this assessment, the department followed the USEPA's Exposure Decision Tree for defining RfD Apportionment and the percentage method to attempt to develop RSCs that were more reflective of exposure experienced by Florida's population. The department attempted to derive RSCs for 10 specific parameters using a combination of Florida-specific, regional, and national level data. One of the greatest challenges was accurately assessing exposure given the sometimes large gaps in available exposure data for state and even regional exposure scenarios. Given, rigorous data requirements and potential uncertainty with capturing the full range of chemical specific exposures, the department concluded the RSCs for our HHC would be the same as EPA's national recommended default values. Exposures to non-carcinogenic compounds is variable and dynamic within a state's population. More rigorous state-specific exposure data that accurately captures exposure variability and inter- and intra-agency collaboration would help state-level environmental regulators to refine RSCs developed for deriving protective human health-based surface water quality standards.

509 The Development of Data-Driven Exposure Distributions for Risk-Based Soil Cleanup Criteria

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There has recently been renewed interest in using probabilistic methods to derive both default and site-specific soil cleanup criteria for protection of human health. Along with this interest has come increasing demand for scientifically defensible exposure distributions. Unfortunately, the exposure data needed to adequately define variability distributions are often limited, and in some cases non-existent. When a distribution for an exposure variable is not available from an accepted source (e.g., EPA Exposure Factors Handbook), or the distributions from accepted sources are not applicable to a site, a risk assessor must determine whether there are data of sufficient quality and quantity available to create a distribution, or if the cleanup criterion is better served by using a point value. The goal of the probabilistic approach should be to increase the accuracy as well as the understanding of complex risk questions. Common problems include: 1) Determining when data are good enough to derive an exposure distribution; 2) Whether, or to what extent, to re-sample data from observations over a limited period of time to reflect the distribution expected with chronic exposure; 3) How to manage uncertainty effectively without invoking a full 2-dimensional probabilistic analysis; and 4) Determining whether sensitive subpopulations and lifestages need to be addressed separately from the general population. Practical examples illustrating each of these problems will be presented, and the related science policy decisions that must be made will be discussed.

510 Deriving Health Human Criteria for Florida using a Probabilistic Approach

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In May of 2016 the state of Florida proposed an update to its human health based water quality criteria. Human health water quality criteria are established at the highest concentration of a pollutant in water that protects against a significant risk of adverse health effects over a lifetime. The criteria should provide adequate protection to the general population over a lifetime of exposures and to special subpopulations (those with high water or fish intake rates or higher sensitivities) that have an increased risk of receiving a dose that would elicit adverse effects. The state's proposed criteria were based, in part, on EPA's 2015 recommendations, but were derived using the probabilistic risk approach (PRA) and Florida-specific information. Florida's approach used national recommended toxicity factors and relative source contributions, and Florida-specific bioaccumulation factors as point measurements in the calculations. Drinking water rate, body weight, and regional fish consumption rates were entered into PRA analysis as distributions. Criteria were set at levels necessary to attain a priori risk management goals. For carcinogens, protective criteria were derived by iteratively conducting the probabilistic risk analysis until the target risk of 10^{-6} at the arithmetic mean of the risk distribution was attained. Additionally, the resulting risks associated with the calculated criteria were assessed to ensure that none of the criteria values produced risks exceeding 10^{-5} at the 90th percentile or 10^{-4} at the extreme upper end of the distribution (e.g., 95th, 99th percentiles, and subsistence fishers). For non-carcinogens, the criteria were derived by running the probabilistic analysis (Monte Carlo) to calculate surface water criteria, and then setting the criterion at the 10th percentile of the Monte Carlo calculated results. Florida's experience with PRA shows that PRA allows regulatory agencies to more accurately assess risks to the target population and sensitive sub-populations than the deterministic alternative. However, Florida's experience also shows that use of PRA adds complexity to an already complex issue, which can hinder public support.

511 Inclusion of Physiological, Metagenetic and Genetic Susceptibility Distributions in Exposed Human Populations for Probabilistic Risk Assessment

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The U.S. Environmental Protection Agency (EPA) is responsible for ensuring the protection of the water quality of the nation's waters by reviewing and approving or rejecting and issuing for each state an enforceable Water Quality Standard (WQS) for each priority substance or mixture in each pollutant category for each fresh and salt water body based on a set of narrative and numerical Water Quality Criteria (WQC) to protect various human uses of those waters. Unless affirmatively demonstrated otherwise by a Use Attainability Analysis, all waters are assumed to be able to support and are protected for fishable and swimmable uses by humans. This requires that the fish and shellfish populations supported by those waters can be safely consumed by humans. A WQC to protect human health is derived by translating a reference dose (RfD) or cancer potency factor (CPF) with an ample margin of safety into an equivalent water concentration based on a set of reference human exposure assumptions. The EPA guidance for WQC derivation uses national average exposure assumptions, but states may choose state average exposure assumptions if they are more protective of human health than the national average exposure assumptions. The derivation of WQC using probabilistic risk assessment (PRA) generally focuses on the probability distributions functions (pdfs) for body weight, eating rate as a function of age, sex, and body weight, the proportions of various fish and shellfish species in the diet by age, sex, ethnicity, and region, and contaminant concentrations in each fish and shellfish species, without or with conditional influences of size or age, while holding the RfD or CPF constant. This is a one-sided approach to PRA. To comport with the recommendations of the National Academy of Sciences to EPA on the regulatory applications of risk assessment, this presentation considers the other side of the equation: the known distributions of physiological, metagenetic, and genetic susceptibilities to carcinogenic toxic effects endpoints by gender, race and country of origin and those inferable from the cumulative pdfs for age of onset. This is a more rigorous way to derive an ample margin of safety (AMOS) to protect the representative most sensitive citizen. This will avoid a class action lawsuit based on government-sanctioned minority discrimination and ensure an AMOS for states that use the implicit approach to calculate Total Maximum Daily Loads for water quality-impaired waters.

Advances in Exposure Modeling: Bridging the Gap Between Research and Application

512 Lifetime Exposome Modeling

O. Jolliet, Univ of Michigan

"Tell me when and where you lived and worked, what you consumed and ate… and I will tell you who you are (your exposome!). In the era of high-throughput biology and biomedicine, genome-wide associations need to be complemented by exposome wide associations, thus emphasizing the importance of developing high-throughput exposure strategies. Complementary to biomarker oriented approaches, this study explores how recent databases enables us to trace and predict the lifetime exposure of individuals to thousands of chemicals based on the time evolution of their home location, economy sector workplace, consumption patterns, and eating habits. We use the measured concentrations from the OSHA Chemical Exposure Health Database to characterize exposure to 250 chemicals per blue collars hour worked in 880 NAICS industrial sectors. These occupational exposures are confirmed by significantly higher biomarker levels measured in NHANES for blue collar workers compared to the rest of the population. Coupling of household product databases with near-field exposure models and near-field modeling of product intake fractions (PiFs) provide first estimate of ranges of exposure to cosmetics, building material or other consumer products. A spatialized multiscale multimedia model (Pangea) provides improved resolution to better assess environmental and food contaminants. This presentation will show how these recent developments in exposure data and modeling for ambient (food, drinking water), occupational and consumer product environment enables us to predict lifetime exposure across multiple chemicals. The approach will be illustrated through a case study in Michigan that has enabled us to determine the lifetime dioxin exposome of hundreds of habitants in a contaminated area, based on their dioxin blood measurements using a phamacokinetic based approach. The study identifies as major factors of influence the number of years lived in the contaminated area, the distance to the local incinerator and their consumption of potentially contaminated food. On a wider scale, we will finally shortly discuss how the exposome relates to the age response of multiple biomarker exposures measured in NHANES.

513 Spatial improvements leading to advances in down-the-drain chemical exposure modeling with iSTREEM® 2.0

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iSTREEM® ("in-stream exposure model") is a publically-available web-based model (www.istreem.org) that estimates down-the-drain chemical concentrations in waste water treatment plant (WWTP) effluents, drinking water intakes (DWI), and in streams impacted by domestic waste water effluent across the continental U.S. and a number of watersheds in Canada under mean annual and low-flow (7Q10) conditions. Major upgrades to the model's underlying data were made by incorporating higher-resolution and more current spatial datasets, leading to the release of iSTREEM® 2.0. The presentation provides an overview of the development of iSTREEM® 2.0, including how specific data needs were addressed and major assumptions considered in developing the model. The model river network was upgraded to a higher-resolution hydrologic dataset based on the USEPA and USGS NHDPlus version 2, which constitutes about 228,000 river segments totaling 243,000 river miles across continental U.S. For all the river segments, estimated mean annual flows were derived from NHDPlus, but low flows (7Q10) were exclusively developed for iSTREEM® 2.0. WWTP and associated facility level information were derived from the most recent USEPA Clean Watershed Needs Survey 2012 dataset, which includes about 13,000 facilities accounting for a total population of 175 million and effluent flow of 25,000 MGD. WWTP facilities were associated to the river network by applying techniques developed by USEPA. Enhancements to the model algorithm has made it possible to run the simulations efficiently and examine chemical exposure at a detailed spatial scale over a large geography (river basins or U.S.). Model simulation results are accessible to users in tabular (MS Excel) and spatial (MS Access) data formats for easy interpretation and further customization. A case study comparing prior version of the model and latest iSTREEM® 2.0 for the U.S will be presented to examine the impact of recent upgrades to model results – with focus on the national distribution of flows (mean and 7Q10's), effluent PEC's, water use, dilution factors, and receiving surface water PEC's. The developments to iSTREEM® improves its utility as a tool to support environmental exposure assessments by a variety of users for environmental risk assessments across multiple commodity groups (personal care products, pharmaceuticals, food additives, pesticides, etc.).

514 Impacts of Hydroelectric Power Expansion on Methylmercury Exposures of Northern Indigenous Communities

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Renewable energy plans across North America depend on untapped hydroelectric resources across Northern Canada. Hydroelectric reservoirs accelerate production of methylmercury (MeHg) resulting in enhanced accumulation in fish and wildlife and pose risks to indigenous populations that rely on local foods. The magnitude of MeHg increases in local food webs is affected by the quality and quantity of organic carbon in the flooded reservoir. We use soil organic carbon data and the watershed characteristics of planned hydroelectric development areas across the Canadian North to develop a screening model for peak MeHg increases expected in each system. Over 90% of new capacity impacts the traditional hunting environments of local indigenous populations. We use extensive biogeochemical data from the region surrounding the Lower Churchill River in Labrador, Canada and human exposure information from three local Inuit communities to quantify increase in MeHg exposure due to flooding associated with hydroelectric facilities. More than half of the total exposure increases among this population are expected to fall on individuals who are in the top 10% of exposure levels prior to flooding. Among the Labrador Inuit, hydroelectric development is expected to roughly double the number of individuals exceeding EPA and Health Canada reference doses for MeHg. However, there may be as few as 20% more individuals exceeding these thresholds if topsoil is removed, as this is the main reservoir of labile organic carbon.

515 Assessing Exposure and Ecotoxicological Impacts in the State of Qatar

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Exposure science and ecological risk assessment continues to develop and evolve in the State of Qatar. Efforts have been taken to utilize appropriate tools and methodologies developed in North America, Europe as well as other relevant hot and arid countries. In many cases, however, the extreme environmental conditions (e.g., high water temperature and salinity) and differing ecosystem structures (e.g., warm water species such as corals) require adaptation of the typical approaches and tools. At EMRQ we are developing an integrated approach to assess discharges, fate, exposure and ecotoxicological impacts in the marine environment. Our initial focus is on the Liquefied Natural Gas (LNG) industry which is important to Qatar and other global regions; and increasingly so for North America, both in the United States and Canada. We have worked closely with our industry partners to robustly quantify discharges and model transport/fate using a spatially and temporally resolved hydrodynamic model combined with laboratory-based data. We also incorporate measured field data for evaluation of these tools and to assess ecological impacts. In the laboratory we have developed ecotoxicology protocols for reference species applicable to Qatari waters as well as dose-response relationships across various trophic levels and key contaminants. Current efforts include the continued development and application of an EcoRisk modeling framework which ties together these multiple components and environmental stressors into a tool that can be used by industry and government to assess ecological risk related to current discharge and exposure scenarios and also for future predictive and planning purposes. This presentation will include recent updates to the modeling framework and application to an environmentally-relevant case study.

516 Assessing the influence of secondary organic versus primary carbonaceous aerosols on long-range atmospheric PAH transport

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We use the chemical transport model GEOS-Chem to evaluate the hypothesis that atmospheric polycyclic aromatic hydrocarbons (PAHs) are trapped in secondary organic aerosol (SOA) as it forms. Trapping of PAHs in SOA would prevent evaporation to the gas phase and protect PAHs from degradation; this could potentially explain why model-estimated PAH particulate fractions are frequently lower than those observed in the atmosphere. We test the ability of three different partitioning configurations within the model to reproduce observed total concentrations in the midlatitudes and the Arctic as well as midlatitude gas–particle phase distributions. The configurations tested are (1) the GEOS-Chem default configuration, which uses instantaneous equilibrium partitioning to divide PAHs among the gas phase, a primary organic matter (OM) phase (absorptive), and a black carbon (BC) phase (adsorptive), (2) an SOA configuration in which PAHs are trapped in SOA when emitted and slowly evaporate from SOA thereafter, and (3) a configuration in which PAHs are trapped in primary OM/BC upon emission and subsequently slowly evaporate. We also test the influence of changing the fraction of PAHs available for particle-phase oxidation. Trapping PAHs in SOA upon formation and protecting against particle-phase oxidation (configuration 2) better matches observed remote concentrations compared to our default configuration (configuration 1). However, we find that simulating adsorptive partitioning to BC is necessary to reproduce the magnitude and seasonal pattern of gas–particle phase distributions. Thus, the last configuration (configuration 3) results in the best agreement between observed and simulated concentration/phase distribution data. The importance of BC rather than SOA to PAH transport is consistent with strong observational evidence that PAHs and BC are co-emitted.

517 Sorptive capacities of leaves for organic pollutants measured using passive dosing: Lipid characterization and passive dosing experiments

D.J. Bolinius, M. MacLeod, A.H. Kierkegaard, F. Iaderasta, J. Holmbäck, Stockholm Univ / Dept of Environmental Science and Analytical Chemistry ACES; A. Jahnke, Helmholtz Centre for Environmental Research – UFZ / Cell Toxicology

Leaves play an important role in the cycling of semi-volatile organic pollutants as a result of their large surface area and lipid-rich cuticle. Semi-volatile pollutants that are scavenged from the atmosphere by leaves can be transferred to the soil through the shedding of waxes and litter (Horstmann and McLachlan 1998), where they will either be trapped or re-released to the atmosphere upon decomposition of the plant material. Most multimedia chemical fate models use either an octanol-equivalent model for leaves (e.g., BETR, MacLeod et al. 2011) or reported values from the literature for specific species under the assumption that all plant species have the same sorptive capacity (e.g., CoZMo-POP, Wania et al. 2006). It has been shown, however, that the sorptive capacities of different plant species can vary considerably (Kömp and McLachlan 1997). In this study we modified an existing passive dosing system to measure the sorptive capacities of the solvent extractable organic matter (EOM) of a wide variety of leaves including; Norway spruce, douglas fir, red oak, common reed, European beech, rhododendron and European alder. Lipids present in the EOM were characterized by ^{1}H-NMR spectroscopy in combination with LC-MS and LC-ELSD. While the previous passive dosing setup via headspace reached equilibrium for cyclic volatile methylsiloxanes within 9 days, the kinetics of equilibration were slower for chlorobenzenes and low-chlorinated PCBs. Introducing a fan into the lid of the passive dosing system enhanced the kinetics such that equilibrium could be reached for chlorobenzenes, and estimated by extrapolation for several PCBs. Our measurements provide sorptive capacities of EOM from a range of species and provide additional data about variability between plant species that can be compared with existing literature data to support improved parameterization of multimedia models.

518 Characterizing the Health Impact of Chemicals from Use of Dishwasher Detergents

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Dishwasher use is a common cleaning activity and can lead to detergent residues remaining on the dishes as well as being emitted during a run. The purpose of the current study is to 1) determine the mass intake and uptake of the chemicals in dishwasher detergents as well as 2) quantify their impact on human health by calculating the characterization factor. Sodium carbonate, pentasodium tripolyphosphate, formaldehyde, sodium perborate, and propylene glycol were selected for study based on their potentially harmful properties. The residual mass of the chemicals on the dishes was calculated by using the air to water partition coefficient. The mass uptake was estimated by taking 30% of the residual mass. A mass balance that models the dynamics in the kitchen and the rest of the apartment was developed in order to determine the cumulative mass of the chemicals released during a run of the dishwasher, so that a mass intake can be estimated. The body burden is estimated as the total mass of the chemical intake and uptake. Only the characterization factor due to ingestion was calculated due to data availability in the LD50 for ingestion, which is the dose at which death is observed in 50% of the population. Finally, seconds of life lost was calculated using data pertaining to percent composition and assumptions of 70 years in a human lifetime and the use of a dishwasher 35 times in a year. Of the studied chemicals, the more volatile chemicals such as sodium carbonate, propylene glycol, and formaldehyde resulted in a higher intake and overall body burden, while the more hydrophilic chemicals such as pentasodium tripolyphosphate and sodium perborate resulted in a higher uptake but a lower body burden. The amount of lifetime lost due to ingestion is ranked as follows: propylene glycol (88 s), sodium carbonate (0.58 s), formaldehyde (0.44 s), pentasodium tripolyphosphate (0.33 s), and sodium perborate (0.12 s). The analysis shows that chemicals with a low LD50 and high percent composition will result in a high negative health impact, which will be helpful to develop a ranking of detergent chemicals based on high exposure and high impact.

519 A Novel Spatial Aquatic Food-Web Bioaccumulation for Bridging Field Study Data and Regulatory Decision Making

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Increasingly, the value of field data is being recognized in the regulatory evaluation of the bioaccumulation properties of commercial chemicals as laboratory measurements of the bioconcentration factor (BCF) and the octanol-water partition coefficient (Kow) regularly fail to identify chemicals of concern. This study develops, tests and applies a novel, spatially based food-web bioaccumulation model to support empirical monitoring programs and the application of field monitoring data for making regulatory decisions regarding the bioaccumulation behavior of chemicals. The study reports on (i) the main features of the spatial food-web bioaccumulation ("Multi-Box AquaWeb"); (ii) its testing through a comparison of model estimated and measured TMFs for persistent PCB congeners and biotransformable phthalate esters in a marine aquatic food-web; (iii) the application of the model for making assessments of the bioaccumulation behavior of chemicals in food-webs. A model sensitivity analysis shows that species sampling designs that ignore the presence of concentration gradients can misidentify the distribution of chemicals in food-webs. The study shows that accurate measurements of trophic distribution of chemicals are strongly dependent on the characterization of spatial concentration gradients in the study area. Also, an accurate assessment of the food-web distribution of chemicals and the frequently used bioaccumulation metric Trophic Magnification Factor (TMF) are sensitive to concentration gradients and species migration patterns. The Multi-Box AquaWeb model is shown to be a useful tool in the assessment of the food-web distribution and also the TMF of chemicals, specifically in anticipating the effect of spatial concentration gradients on the determination of the TMF; guiding species collection strategies in TMF studies; and interpreting the results of field bioaccumulation studies in study locations that are subject to spatial differences in chemical concentration.

Canadian Oil Sands: Advancing Science in Chemical and Toxicological Characterization, Reclamation and Monitoring – Part 1

520 The toxicity of diluted bitumen to four fish species

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The transport of diluted bitumen (dilbit) from Alberta's oil sands through pipelines occurs across many watersheds in Canada. However, little is known about its potential toxicological impact on different fish species in case of a spill. Embryos of four fish species were exposed to two predominant dilbit blends, Access Western (AWB) and Cold Lake (CLB): Japanese medaka (Oryzias latipes), fathead minnow (Pimephales promelas), yellow perch (Perca flavescens), and rainbow trout (Oncorhynchus mykiss). Static daily-renewal exposures were conducted through water-accommodated fraction (WAF) and chemically enhanced fractions (CEWAF, Corexit®9500 as dispersant) from fertilized eggs until hatch. Hatched fish were assessed for malformations and gene expression analysis. GC-MS and fluorescence methods revealed that the array of polycyclic aromatic hydrocarbons (PAHs) was similar between blends, the proportion of individual alkyl PAHs (APAHs) varied between WAF and CEWAF. Exposure to higher concentrations of dilbit increased the prevalence of malformations in all four species, but with species-specific developmental malformations, suggesting that some species are more sensitive to chronic dilbit exposure than others or accumulate more hydrocarbons. Related oils and compounds have shown to induce malformations by increasing oxidative stress. Dilbit has also been found to increase oxidative stress in fish species. Cytochrome P450 (cyp1a) mRNA levels were more than 10-and 100-fold higher in dilbit-exposed medaka and rainbow trout, respectively. Gene expression patterns observed for other biomarkers involved in phase I detoxification (ahr, arnt) and oxidative stress (cat, gpx, gst, gsr, g6pdh, hsp70)pathwayssuggest that fish respond to PAH toxicity in a concentration-dependent and species-specific manner. Comparing responses among fish species allows for development of a suite of biomarkers that indicate sensitivity of economically- and environmentally-important fish species to dilbit toxicity.

521 Review of methods for oil toxicity testing and implications for assessing dilbit toxicity

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The production of diluted bitumen (dilbit) in Canada may soon exceed the transportation capacity of existing oil pipelines, which has stimulated several proposals to expand or build new pipelines to transport dilbit across Canada. Ecological risk assessments (ERAs) for potential dilbit spills have relied heavily on widely-used toxicity test methods with conventional crude oil to assess the risks of dilbit spills to aquatic species. Dilbit toxicity tests require gradients of dilbit concentrations in water, prepared from mechanical and chemical dispersions of oil in water. Methods for preparing Water Accommodated Fractions (WAFs) and Chemically Enhanced Water Accommodated Fractions (CEWAFs) have been developed by CROSERF (Chemical Response to Oil Spills: Ecological Effects Research Forum) and are widely used in toxicity testing. These standardized protocols were developed to allow the comparison of dispersant and dispersed oil toxicity among types of oil, dispersants, and aquatic species. The assumption is that the methods are appropriate for estimating dilbit toxicity and that the data will meet the needs for site-specific (i.e., realistic) ERAs. However, tests of the toxicity of different dilbit products and hydrocarbon analyses of test solutions have demonstrated the need for modifications to the standardized method. The unique physical and chemical properties of dilbit, particularly their rapid weathering, high viscosity, and adhesiveness, affect the extent to which the components of dilbit are incorporated into test solutions by the standard methods. There has been insufficient investigation of how the physical and chemical characteristics of dilbit products interact with procedures for solution preparation to affect the concentration and composition of test solutions and the outcome of toxicity tests. We will review the existing methods for preparing, analyzing, and testing the toxicity of WAFs and CEWAFs of dilbit to aquatic biota to assess their suitability to estimate the toxicity risks of dilbit spills.

522 Potential sources of methylmercury in snowpacks and tailings ponds of the Athabasca Oil Sands Region, Alberta, Canada

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Methylmercury (MeHg), a neurotoxin that biomagnifies through food-webs, is a contaminant of concern in the Athabasca Oil Sands Region (AOSR) of Alberta, Canada. Previous research demonstrated that winter loadings of MeHg in snowpacks increased with proximity to the major developments (0.66 ng/m^2 200 km away to 15.4 ng/m^2 nearby) and that the majority of the MeHg was particle bound (pMeHg) (Kirk et al. 2014, ES&T, 48, 7374). To determine if the pMeHg was produced by methylation of inorganic Hg (HgII) within snowpacks, we performed a series of in-situ incubation experiments using stable isotopes of HgII at four sites known to vary in pMeHg loadings. Those experiments demonstrated that there is net methylation of Hg(II) in snowpacks; however, rates were very low (k_m = 0.001 – 0.004/d), suggesting that in situ snowpack methylation is not a major source of MeHg to snowpacks. We also quantified concentrations of MeHg on particles themselves, as well as examined particle size, morphology, and elemental composition. Interestingly, although MeHg snowpack loads increased with proximity to the major development area, pMeHg concentrations themselves significantly decreased with proximity (0.53 (near) –10.35 (far) ng/g; R^2= 0.64, p< 0.0001). These results suggest that a large mass of particles is deposited to snowpacks near the major developments; these particles are not rich in MeHg relative to particles found further away. Although little is known about the sources of pMeHg to AOSR snowpacks, it was recently suggested fugitive petcoke dust may be an important source of wind-blown PAH contaminant deposition to the region (Zhang et al. 2016 ES&T, 50, 1711). MeHg concentrations in three petcoke samples were examined and were lower (0.02 ± 0.01 ng/g) than on particles in snowpacks (2.47 ± 0.56 ng/g), suggesting that petcoke is not an important source of MeHg to the region. Another potential source of MeHg to AOSR aquatic ecosystems is tailings ponds (TP), which may seep into the Athabasca River and tributaries. We therefore quantified concentrations of MeHg in TP water of four different ponds of varying size, age, and chemical composition. The four TP varied greatly in MeHg concentrations, from very low concentrations of 0.01 ng/L to relatively high concentrations of 0.50 ng/L. Potential factors driving MeHg sources in TP are being examined, including pH, nutrients, oxygen, and DOC, all of which are known to affect MeHg production in aquatic ecosystems.

523 Spatial and Temporal Variation of Trace Metals in the Athabasca Oil Sands Region Based on Dated Lake Sediment Cores

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Assessments of the environmental impacts of the Athabasca oil sands development in northeastern Alberta have noted the lack of long-term data and limited information on pre-impact conditions. The extraction of bitumen from the Athabasca oil sands – one of the largest energy deposits in the world – began in earnest during the 1960s. A steady increase in production has accelerated the release of environmental contaminants, including various trace elements known to be toxic at low concentrations. Of particular environmental concern are the atmospheric loadings and distributions of trace elements associated with oil sands surface-mining and processing activities. Establishing the magnitude of change in the regional deposition of trace elements requires knowledge about conditions prior to the onset of large-scale extraction activities. Here, we will present results from 23 dated sediment cores from lakes located across the Athabasca oil sands region. Lakes were cored between 2011 and 2013 as part of the Joint Canada-Alberta Oil Sands Monitoring Program. Lakes with small, undisturbed watersheds were targeted in order to better reconstruct atmospheric inputs through time. We categorized each sites based on its distance from the geographic center of mining operations as being near- (50 km). We observed increasing inputs of vanadium and lead, two key indicators of oil sands development, to near- and mid-field lake sediments after mining operations began in the 1960s. Other metals (e.g., Hg) are characterized by earlier increases that are of similar magnitude among near-, mid-, and far-field sties, suggesting non-oil sands sources are more important.

524 An omics platform for the advancing of the environmental monitoring program in the Alberta oil sands region

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In Northern Alberta, Canada, the production of bitumen from open-pit oil sands mining requires 2-3 barrels of water for every barrel of bitumen produced. Large volumes of oil sands process-affected water (OSPW) are stored in large tailings ponds so that it can be recycled into the extraction process. Despite the water recycling efforts (80 to 95%), in 2012 the use of fresh water was approximately 187 million m^3 (Canadian Association of Petroleum producers 2013); which is about 40% of the City of Toronto's annual water consumption. Moreover, due to the large water volume used daily, tailings ponds are growing in volume and number, and in 2011 covered approximately 170 km^2. There are concerns that OSPW leaches from tailings ponds into groundwater, or into river water, but proving this is complicated by the fact that natural water can contain many of the same chemicals as OSPW (e.g. Naphthenic acids). As a consequence, there is an ongoing need to improve environmental monitoring in the Athabasca region. Due to the complex nature of OSPW, an 'omics' approach to interrogate and interpret the large volume of data generated during the analysis of water samples by LC-Orbitrap-MS (operated with resolution power from 120K to 480K) was developed. The approach was applied to river water samples collected in the Alberta oil sands region. Results indicate that the approach extends monitoring capabilities from the "classical" naphthenic acids to the inclusion of ionisable compounds with molecular formula $C_cH_hN_nO_oS_s$. It has recently been postulated that compounds belonging to this general class contribute to the toxicity of OSPW. Thus, obtaining information about these compounds will enable regulators and environmental managers to improve monitoring and reclamation programs in the region.

525 A spatial and temporal assessment of contaminants in otoliths from the Lower Athabasca region

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Potential environmental impacts of anthropogenic water use and contaminant emissions from oil sands development in the Lower Athabasca region has gained public and scientific attention. Fish health and contaminant loads are of particular interest because of perceived increases in fish deformities, and complaints about unnatural tasting fish since the onset of industrial development in the region. Although the presence of bitumen-associated contaminants has typically been attributed to industrial activity within the public and media, the relative roles of natural seeps, which contribute salts and complex dissolved organic mixtures to the watershed, and industrial emissions of similar compounds are largely unknown. The aim of this research is to assess water quality along the Athabasca River, and its impact on fish. Trout perch, Percopsis omiscomaycus, were sampled from 31 sites along the Athabasca and Clearwater Rivers in the Fort McMurray region. The sites were categorized as reference sites, and potentially contaminated sites from either anthropogenic or natural sources. We used laser-ablation inductively coupled mass spectrometry (LA-ICP-MS) to analyse otoliths to assess spatial and temporal variation in concentrations of a large suite of elements. The suite of elements includes some present in water exclusively in ionic form (e.g. Na, Rb, Ca, Sr), some present in colloids (e.g. Al, Sc, Th), nutrients (e.g. Fe, Se), some known to be enriched in bitumen (e.g. V, Ni, Mo), and some potentially toxic "heavy metals" (e.g. Ag, As, Cd, Pb, Sb, Tl). In general, many of the elements included in this study were found in low concentrations and were challenging to measure. Concentrations may reflect the chemical characteristics of the elements (e.g. affinity for carbonate mineral formation) or biological function. Results of this study demonstrate whether and how potential contaminants and other elements that are present in varying concentrations along the stretches of river are present in fish inhabiting the river.

526 Fish Health in the Alberta Athabasca Oil Sands, Developing Baseline to Assess Future Change

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As part of the Joint Canada-Alberta Oil Sands Monitoring Program (JOSM), fish health within the Athabasca River watershed is being evaluated using methods developed for the Canadian Environmental Effects Monitoring Program. Fish health assessments will be integrated with assessments of benthic invertebrate communities, water and sediment chemistry, toxicology and atmospheric deposition in order to evalute ecosystem health. Data will provide a baseline against whcih future changes in fish health will be judged and compared to historical studies to assess change. Information will be used to develop site-specific cumulative effects monitoring approaches and will contribute to the development of better predictive capabilities for oil sands environmental impact predictions. Additional physiological endpoints are also being evaluated in fish from these sites in attempts to better understand mechanisms of action and potential source identifiaction of changes. On the Athabasca mainstem, white sucker have been selected as a large bodied sentinel species and trout perch as a small bodied species. Walleye are also being used for fish contaminant monitoring within the mainstem as they are consumed by resident populations. Where possible our study design collects fish at sites off the oil sands deposit (reference), at sites within the natural deposit but upstream of development and at sites downstream of development so that fish are either unexposed, exposed naturally or exposed to a combination of natural and anthropogenic inputs. Design of the program is to look for differences, confirm the differences and to use developed critical effect sizes to make decisions on steps forward. We will discuss fish studies to date with recommendations on how to proceed with the monitoring program moving forward.

527 Where do we go from here? Lessons from 25 years of Environmental Effects Monitoring (EEM) in Canada

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Monitoring shouldn't be difficult, but it is. And despite the difficulty of detecting and attributing cause, its importance has not diminished. A Federal EEM program was developed in the early 1990s for determining the efficacy of guidelines regulating the discharge of pulp mill effluents and (later) metal mines. After 25 years of developing consistent monitoring for large industries in Canada, many lessons can be gleaned for application in other areas. Although implicitly included in the development of EEM, Adaptive Monitoring has recently been formalized and offers an opportunity to develop and operate meaningful monitoring programs. An Adaptive Monitoring program is characterized by hypothesis-driven questions linked to conceptual effect pathways, evolving through trigger-driven feedback loops that connect tiers of monitoring. Triggers describe background variability and notify us when an unexpected change has occurred. Tiers represent an escalation of effort or attention towards those unexpected observations. Tiering allows the simultaneous operation of both broad non-specific tools and targeted sampling. Other recent improvements include the development of adverse outcome pathways as simple expected effects pathways. A logical question in an AOP process as applied in monitoring is: if we see a change in some physiological indicator, and the difference matters, what will change next? These ideas and a more detailed discussion on improving environmental monitoring will be presented.

Thinking Outside the Laboratory Box: An Ecological Approach in Tackling Ecotoxicological Problems

528 Exploring the intersection between ecosystem ecology and ecotoxicology: Developing the field of ecosystem toxicology

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Exploration of ecosystem-level phenomena within the field of ecotoxicology have been somewhat limited in the past, but this line of inquiry is receiving increasing attention from scientists across multiple disciplines. We convened a special session at SETAC 2015 to explore the intersection between ecosystem ecology and ecotoxicology and to take the initial efforts to delineate what we have identified as a new subdiscipline: ecosystem toxicology. This session highlighted that combining environmental chemistry, ecotoxicology, and ecosystem science is necessary to better understand contaminant exposures and their effects on the environment. Ecosystem toxicology provides a new platform for investing the influence of contaminants in the environment. We propose that ecosystem toxicology could focus on 4 broad themes: 1) Exploring both how ecosystems influence how chemicals behave once they enter the environment, which relies strongly on collaboration between environmental chemists and ecosystem scientists. 2) The influence of chemicals on ecosystem functions, e.g. primary and secondary production, nutrient cycling, etc. This requires investigation by ecosystem ecologists who typically measure these variables in ecosystems. 3) The flux of contaminants through ecosystems, which requires combining ecological measurements, e.g. organic matter fluxes, with measures of chemical concentrations in biotic and abiotic compartments. 4) That context, from the species to the ecosystem, is fundamental to the influence of contaminants in the environment and that responses to contaminants should be studied across a range of species and environmental contexts. We argue that these areas are ripe for collaboration among toxicologists, environmental chemists, and ecosystem scientists and that these collaborations will provide new insights into the influence of contaminants on the environment.

529 Aquatic agrochemical pollution increases the risk of human schistosomiasis

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Agrochemical use is predicted to increase 2- to 4-fold by 2050 to satisfy the food demands of growing human populations, with much of this increase expected to occur in tropical and sub-tropical developing countries. At the same time, the expansion of irrigation-related agricultural activities has already been associated with the emergence of schistosomiasis, a neglected tropical disease caused by a flatworm parasite that is transmitted from aquatic snails to humans. This association is multidimensional and key pathways have been poorly characterized, including the role of agrochemical pollutants, which might increase numbers of the snail intermediate hosts by affecting the algal food resources and predators of snails. In a series of field mesocosm experiments, we show that a common insecticide, herbicide, and fertilizer, individually and as agrochemical mixtures, increased population densities of infected snails, thereby increasing production of parasite infective stages. Our mesocosm results showed that some agrochemicals caused a "bottom-up effect," increasing the growth of the algae snails eat, and others a "top-down effect," decreasing densities of snail predators through direct chemical-induced mortality. Mathematical models parameterized from the mesocosm and LC50 experiments and fit to schistosomiasis epidemiological data from Africa suggest that these observed effects of agrochemicals could significantly increase the risk of human schistosomiasis transmission. Separate mesocosm experiments have identified agrochemicals that do not yield a substantial increase in infected snail abundance; these represent candidate chemicals that might be useful for meeting the food demands of growing human populations without increasing human schistosomiasis. Our results highlight the value of mesocosms, models, and community ecology theory for addressing local to global problems in ecotoxicology and public health.

530 Are fungicides a threat for the functioning of heterotrophic systems?

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Fungicides have the potential to affect inter alia heterotrophic organisms and the functions they provide via multiple pathways. In this study we will discuss the impact of organic and inorganic fungicides on leaf associated biofilms, in particular aquatic fungi, and how these modifications may modulate the quality of leaf material for detritivorous macroinvertebrates. With food selection assays it was, for instance, shown that the inorganic fungicide copper increased the palatability of leaf material for the leaf-shredding amphipod Gammarus fossarum. This preference was attributed to an increased fungal biomass accrual and a shift in the fungal community favoring species considered as more palatable for this amphipod. When, however, fed with these preferred but copper contaminated leaves over multiple weeks, Gammarus showed impairments in growth and energy reserves indicating long-term impacts in the population development of this species. Organic fungicides, in contrast, favored leaf associated fungal species, which are considered less palatable for gammarids, and thus – along with a repelling effect of these substances – induced a rejection of this leaf material. At the same time, this leaf material induced only limited effects in the growth of gammarids indicating, despite strong effects on food selection, low impairments in population development in the long-term. These insights, thus, suggest that the food choice of selectively feeding detritivores may not be predictive for implications on their physiological level, which is supported by similar results for larvae of a caddis fly. It was, nonetheless, shown that fungicides – despite being able to induce toxic effects via waterborne exposure – can modify the physiological fitness of detritivores via bottom-up directed effect pathways at field relevant levels.

531 Whole ecosystem experiments to assess effects of glyphosate and fertilizers on wetland communities

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Glyphosate-based herbicides are some of the most commonly used herbicides in the world. They are generally considered to have a low direct toxicological risk to animals, but few studies have attempted to investigate the ecosystem-level effects of formulated glyphosate products either alone or in conjunction with other likely co-occurring environmental contaminants. Small wetlands are ubiquitous in agricultural landscapes, and have considerable potential for contamination by glyphosate herbicides and fertilizers. Twenty four split wetlands in New Brunswick, Canada, were used to examine the effects of two target concentrations of glyphosate-based herbicides with (G+F) or without (G) fertilizers on wetland macrophytes, plankton, macroinvertebrates and amphibians (n=6 wetland halves/treatment). Wetlands were treated twice with Roundup Weathermax® and four times with nitrogen and phosphorous to mimic agricultural exposures, and comparisons were made between treated and non-treated wetland halves. Initial treatment concentrations across ponds averaged 835 mg acid equivalents (a.e.)/L in the high G and G+F ponds and 27 mg a.e./L in the low G and G+F ponds and glyphosate half-lives in the water column averaged < 1 day. An immediate decline in phytoplankton edible carbon (by 8%) and zooplankton community similarity (by 27%) were observed in the G+F but not G only treated ponds. No short-term effects were observed on macroinvertebrate emergence or composition across any treatments. In contrast, after the macrophyte communities declined (up to 30%) from herbicide treatments and glyphosate was no longer detected in the water column, zooplankton richness and abundance in the G+F treated systems and macroinvertebrate emergence in all wetlands but the high G treatment increased. Wood frog larvae (Lithobates sylvaticus) on the treated sides of wetlands were slightly larger ($< 10\%$) than those on the control side, but no effect on survival or development was observed. The abundance of green frog larvae (Lithobates clamitans) was higher on the treated sides than the control sides of wetlands in the herbicide and nutrient treatments. Overall, direct effects of the herbicide treatments were transient and seen only in wetlands also treated with fertilizers whereas longer-term indirect effects were evident in the invertebrate communities and amphibian populations, indicating the value of assessing multiple stressors in whole-ecosystem experiments.

532 Effects of antihistamine on invertebrates and carbon and nutrient recycling in streams

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A wide variety of pharmaceuticals are found in many aquatic ecosystems all over the world. Groups of pharmaceuticals of particular interest for potential ecological effects have traditionally been EDC:s (endocrine disruption compounds) and anti-depressant/anxiolytic drugs, since they are

designed to alter human physiology and/or behavior. There is a growing awareness among scientists that behavioral variation and alterations are important for individual performance, ecosystem function, and species evolution. In ecotoxicology it has been recognised that behavioral alterations may be caused by contaminants found in natural systems. Recent studies have shown that both EDC:s and anxiolytic drugs can be found in the environment in high enough concentrations to affect behavior of fish. Very little is, however, known about how/if pharmaceuticals can affect the behavior of other aquatic organisms and how these effects in turn might affect ecosystem processes and functioning. Here I will present results from a sequence of experiments studying effects of the antihistamine fexofenadine on aquatic invertebrates. These studies were then used as a knowledge base for ultimately testing potential effects on important ecosystem processes (i.e. carbon and nutrient turnover). We tested this by investigating if the antihistamine fexofenadine alters rates of leaf litter decomposition in stream microcosms at concentrations 100 times lower than the predicted no-effect concentration. Stonefly larvae, together with natural microbial communities, served as main decomposer organisms on alder leaf litter. Interestingly, concentrations of organic carbon (TOC) and nitrogen (N) were strongly affected, with 20–26 and 24–31% lower concentrations of TOC and N, respectively, in the presence of fexofenadine. I will discuss potential reasons for the pronounced effect on carbon and nutrient turnover and why it could be important.

533 Aquatic insect emergence and pesticide flux from wetlands to terrestrial food webs in the Prairie Pothole Region

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Pesticides can have adverse effects on aquatic insect communities and ultimately impact terrestrial consumers. Little is known about the effects of pesticides either on emergence production of adult aquatic insects or pesticide flux to terrestrial consumers such as insectivorous birds. In particular, a recent meta-analysis of contaminant transfer from larvae to adult insects suggest that some pesticides might also be retained in adult aquatic insects, but no current use pesticides were included. We measured insect emergence and pesticide flux in 2015 from 14 wetlands that varied widely in ponded water permanence and geochemistry (e.g., salinity) in agricultural and grassland land use sites in the Prairie Pothole Region (PPR) of North America. The PPR is covered with a range of wetland types that are a crucial breeding and feeding grounds for migrating birds, most notably waterfowl, embedded in a landscape predominantly used for cropland agriculture. Patterns of damselfly and dipteran emergence differed by land use with more damselflies emerging from grassland and more dipterans from cropland wetlands. However, cropland wetlands did not differ in productivity from the grassland wetlands. Pesticide residues including atrazine, bifenthrin, imidacloprid, fipronil sulfone, and p,p'- DDD were found in adult insects emerging from wetlands in all land use types. Pesticide tissue concentrations and flux estimates from wetlands in adult aquatic insects were generally highest from cropped sites. These data will be used to develop predictive spatial models of adult aquatic insect emergence and contaminant flux from wetlands to terrestrial bird consumers.

534 Selenium ecotoxicology in freshwater lakes receiving coal combustion residual effluents: A North Carolina example

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Coal combustion residuals (CCRs), byproducts of coal combustion processes for electricity production, are characterized by elevated concentrations of toxicologically important contaminants including arsenic, cadmium, manganese, and selenium. Via permitted effluent streams, CCRs are chronically released to freshwater systems and accumulate to enriched levels in organisms at the base of the food web. Recently proposed changes for CCR handling and disposal prompted interested in the legacy effects of CCRs in lentic freshwater systems where prolonged water and contaminant retention times lead to sustained impacts on system biota. In the current study, six lakes in North Carolina were selected for comparison; three lakes that receive, or have historically received, coal-fired energy facility effluents, and three reference lakes paired on the bases of lake trophic status and geographical proximity. Surface water, sediment pore water, plankton, and three species of fish were collected from each lake and analyzed for CCR concentrations by ICP-MS in order to determine differences between CCR-impacted and reference systems, whether these differences are associated with lake trophic status, and evaluate potential toxicity in freshwater biota. For the purposes of this presentation, selenium ecotoxicology will be emphasized. Overall, CCR-impacted lakes were found to have significantly higher selenium concentrations in both abiotic and biotic compartments. Notably, fish tissues from CCR-impacted sites contained significantly elevated levels of selenium relative to those from reference lakes ($P < 0.05$) regardless of lake trophic status, selenium loading, or the level of hydrological connectivity. However, site-specific factors were found to influence selenium tissue accumulation. For example, muscle selenium concentrations were 6.2, 8.9, and 1.7-fold higher (all $P < 0.0001$) in fish collected from CCR-impacted lakes relative to those from paired reference lakes in the NC coastal plain, piedmont, and mountain geographical regions, respectively. Results from a field-collected plankton feeding and trophic transfer study and from pore water toxicity assays will also be presented. The implications of this work are pertinent to EPA's proposed selenium aquatic life criterion and recently decided final rule on CCR disposal. Funding support: WRRI Grant 15-03-W, NIEHS Award T32ES021432, and EPA FP917801010.

535 Lessons learned in application of field data to addressing ecogoxicological issues

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The use of ecological and related ecotoxicological data from nature in addressing ecotoxicological problems has long been the subject of discussion. For example, early papers on development of contaminant guidelines suggested data from nature were desirable but were too complex to be useful. However, understanding of geochemistry, bioavailability, species-specific biological effects, ecological implications and models that tie these together has advanced greatly in the last three decades. This paper will review several approaches that are poised to be useful in both defining cause and effect and developing guidelines in cases of metal/metalloid contamination. The emphasis will be on lessons learned in early applications of these approaches. One example is the Presser-Luoma Ecosystem Scale Model (PLESM) that ties together the combined influences of loadings, hydrology, speciation in receiving waters, biogeochemical cycling (especially transformation to living and non-living material at the base of the food web), bioaccumulation at the base of the food web, trophic transfer to predators, transfer to gonads and eggs within animals, and toxicity (especially developmental toxicity). Uncertainties in predicting implications of dissolved Se concentrations are reduced by inclusion of robust geochemical and biological field data, especially time series data, and by careful verification of outcomes. The second example is built from published studies from the Clark Fork River, Montana, USA and streams in the historic Southwestern England mining district of the UK. In these cases metal concentrations in tolerant biomonitor species are calibrated against changes in the abundance of metal-sensitive species across multiple, complex metal gradients. This approach provides a practical, operational way to address several of the challenges faced in understanding thresholds of metal effects in streams and rivers; especially when combined with time series studies, multi-stream comparisons and models that tie ecological change to metal concentrations in different media. One of several advantages of these approaches is that they provide a framework for a constructive discussion among different interest groups of all the data available from an ecosystem of interest and thereby facilitate constructive data-based discussions of different policy choices.

Microplastics in the Aquatic Environment: Fate and Effects – Part 2

536 Microplastic mediated transport of persistent organic pollutants in Daphnia magna – an evaluation of a toxicokinetic model using experimental data

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Microplastic (MP) contribution to the bioaccumulation of persistent organic pollutants (POPs) has been recently debated. Experimental studies have largely focused on MP as a vector of POPs to aquatic animals; however, depending on the relative concentrations of contaminants in the MP and biota, MP may also act as a cleaning agent. Kinetic models have taken this bi-directional transport into account but have, so far, only been evaluated for animals with relatively long gut retention times (hours) with high absorption/desorption efficiencies of chemicals to/from MPs. Here, we have evaluated a toxicokinetic model parameterized for Daphnia magna, a suspension feeding primary consumer with very short gut processing times (minutes). The results of a depuration study, where D. magna contaminated with four polychlorinated biphenyl (PCB) congeners (PCB18, 52, 128 and 209) were fed a mixture of clean MP and algae (1:1, MP:algae ratio by dry weight) was used to evaluate the model. The modelling results will be discussed in relation to observed experimental data.

537 Ingestion of microplastic associated with green algae by Daphnia magna and enhancement of PCBs bioaccumulation

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The concern of plastic pollution has recently grown at global scale. Most research on potential effect of microplastics has focused on marine environment. Recent studies have found that the Great Lakes region has the highest concentration of plastic material in any freshwater source. Many areas of the Great Lakes ecosystem have been found to be contaminated with PCBs. Concurrently, it is the largest source of freshwater in the world and therefore instigates a need to address the effect of these plastics. The present study determined the effects of microplastics consumption in freshwater invertebrates, Daphnia magna and enhancement of microplastics on the bioaccumulation of PCBs in D. magna. Daphnia magna was chronically exposed to fluorescent green polyethylene microspheresof size 63µm-75µm at concentrations of 25, 50, and 100 mg/L. Ingestion of microplastics and reproduction of D. magna were measured. The present study found that D. magna ingested significant amount of microplastics. The average concentrations of microplastics in the gut of D.magna were 26.26 and 76.75 pieces/organism at the lowest and highest water concentrations of microplastics, respectively. However, no statistically significant difference in reproductive rate was found for D. magna of control and exposure treatments. Evidence of increased algal production on microplastics compared to control is present. These results suggest that adsorbed algae on microplastics would provide an additional energy source for reproduction performance of D. magna. In the bioaccumulation experiment, D. magna was chronically exposed to PCBs at a concentration of 1µg/L in presence of 1g/L microplastics or without microplastics (control). At the experiment termination, D. magna was collected for tissue PCBs analysis. Results of the bioaccumulation experiment indicate an enhancement of microplastics on PCBs accumulation in D. magna.

538 Can hydrophobic organic chemicals sorbed to microplastics affect aquatic organisms? A review of laboratory studies

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The potential for microplastic particles (MPs) in the marine environment to cause adverse effects on organisms and ecosystems is an issue of public and scientific interest. One area of concern is the potential for MPs to act as vectors for the transfer of hydrophobic organic chemicals (HOCs) present in the environment to the marine food web. This analysis considered only exogenous HOCs that were not intrinsic to the manufacture of HOCs. We summarized the results of laboratory studies that examined the transfer of HOCs sorbed to MPs and effects on aquatic organisms. Most studies that reported adverse effects associated with exposure to MP + HOCs also reported adverse effects for treatments in which organisms were exposed only to MPs, indicating that reported effects may not be solely due to HOCs. Many studies used environmentally unrealistic concentrations of MPs, making extrapolation to natural environments difficult. Finally, we note that many laboratory studies used gradients of chemical activity (e.g., contaminated MPs and uncontaminated organisms) that might be higher than would be observed in the field where sources of HOCs exist in various environmental media (e.g., food, water). In summary, we conclude that available data from laboratory studies provide weak evidence that transfer of exogenous HOCs from MPs to marine organisms is likely to be an important route of exposure and contributor to adverse effects to marine organisms. More data are needed to fully understand the relative importance of exposure to HOCs from MPs in comparison to other pathways.

539 Microplastic as a vector of PCB uptake in Daphnia magna

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Microplastic (MP) has been associated with the accumulation of persistent organic pollutants (POPs) through ingestion of contaminated plastics by various organisms. However, adequate experimental observations that can be used to test these theoretical expectations are very rare. We conducted a laboratory study to evaluate MP as a vector of POPs in Daphnia magna, a filter-feeding zooplankton model organism. The MPs were loaded with a mixture of polychlorinated biphenyl congeners (PCBs) of varying hydrophobicity ($\log K_{OW}$ 5.6-8.3), mixed with algal food, and fed to the daphnids during 21 days. The treatments included: control (no PCB and no MP), MP exposure (no PCB and 10-900 MP mL^{-1}, the volume of MP in relation to the volume of algal food was 0.02-1.4) and a combined exposure of PCB and MP (ΣPCB 1.46-131.62 ng mL^{-1} and 10-900 MP mL^{-1}). At the end of the experiment,mortality, total offspring production and somatic growth were analysed in concert with PCB accumulation. The congener specific uptake via MP was compared to PCB bioaccumulation in zooplankton samples from natural environments, laboratory experiments and modelled values. We found that at an intermediate exposure treatment, PCB loaded MP had positive effects on growth and reproduction. Whereas negative effects were observed at the highest exposure level, resulting in ~70% mortality. Congener specific bioaccumulation factors, based on the uptake from MP, were similar to earlier reports from natural environments and modelled values. Therefore, MP-mediated accumulation of PCBs in zooplankton, can be satisfactorily explained by the chemicals hydrophobicity and does not diverge from the uptake via other sources (food and water).

540 Ingestion of microplastics by the freshwater invertebrate Chironomus sancticaroli: effects on PBDE bioaccumulation and the gut microbiome

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Microplastic particles in the environment can associate with persistent organic pollutants (POPs) due to the hydrophobic nature of plastics and organic chemicals. If ingested, the gut environment of an organism may favour desorption of adsorbed chemicals due to gut conditions such as pH and ionic strength as well as the presence of surfactants. Therefore the ingestion of microplastic particles has implications for uptake and bioaccumulation of these chemicals. Furthermore the presence of microplastics and chemicals in the gut of an organism can also influence the gut environment itself. PBDEs (polybrominated diphenyl ethers) are widely used as flame-retardants in products such as textiles and soft furnishings, with the potential to leach into the environment and therefore they can potentially associate with microplastics. The aims of this study were to investigate the ingestion of microplastics by the chironimid (Chironomus sancticaroli) and how microplastics affect PBDE bioaccumulation and the gut microbiome. In order to investigate the ingestion and elimination of microplastics by the freshwater midge larvae Chironomus sancticaroli were exposed tomicroplastic particles (fluorescently labelled nylon; < 50 µm) in sand for 48 hours at a concentration of 1%. Ingestion was measured by fluorescence microscopy after 6, 24 and 48 hours. After transfer to clean sand elimination of microplastics was also monitored after 6, 24 and 48 hours elimination period. In a second experiment chironomids were exposed to microplastics and PBDEs both independently and in combination for 96 hours. Microplastic particles were mixed with quartz sand sediment at 1% concentration. A PBDE mix (containing BDE-47, 99, 100, 153 and PBB-153) was added to the sediment-microplastic mix in glass vessels at six environmentally relevant concentrations (94, 188, 375, 750, 1500, 3000 ng g^{-1}). Artificial freshwater was added to each vessel once the solvent had evaporated. Microplastics were ingested by chironomids over the 48 hour period but microplastics were found to be still present in the gut following the 48 hour elimination period. After the 96 hour exposure there was no mortality observed, either for microplastics or PBDEs. However, the microbiome of the chironomids in the presence of microplastics were significantly different from chironomids not exposed to microplastic.

541 Microplastic Contamination at the Base of the Food Chain

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Microplastics (plastics < 5 mm in size) are reported in freshwater and marine ecosystems worldwide, and potential impacts on the food chain are beginning to be recognized. Lanternfishes (Myctophidae), with their global distribution at the base of the food chain, are of both commercial and ecological significance. In the present study, lanternfishes collected from the Pacific and Atlantic Oceans were analyzed to assess microplastic ingestion and contaminant uptake and accumulation. Fish were collected during an August 2009 Project Kaisei North Pacific Gyre expedition and during a November 2010 5Gyres Institute South Atlantic sampling cruise. Lanternfishes, captured during each of the voyages, were analyzed for presence and concentrations of persistent organic pollutants and plasticizers in their tissue. Kaplan-Meier (KM) sums of PCB concentrations were correlated to AP and BPA concentrations in fish tissues. The digestive tracts of individual fish exhibiting highest tissue contaminant loading were dissected and microplastics removed using pulsed ultrasonic extraction. Microplastic particles from both the Atlantic and Pacific Ocean fish guts were examined for size, morphology, and chemistry using optical microscopy, scanning electron microscopy (SEM), Fourier transform infrared spectroscopy (FTIR) and Raman to determine plastic type. Polyethylene(PE) and polypropylene(PP) microplastics were detected in fish stomachs from both locations. Additionally, Raman detected the presence of microplastic/fatty acid conjugates in the Atlantic Ocean fish stomach samples. In addition to the microplastics, the fish stomach contents contained a variety of particles easily misidentified as microplastics by optical examination. Ingested particles appeared to consist of both degradation products from larger plastic pieces as well as manufactured microbeads and microfibers. Important lessons on the aquatic distribution, characteristics and behavior of microplastics and their impact on the food chain can be learned from this study as well as requirements for methodologies to accurately identify plastic versus non-plastic particles.

542 Assessment of microplastic ingestion in commercial fish species Engraulis encrasicolus and Sardina pilchardus in the Western Mediterranean Sea

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Microplastics in the marine environment are ubiquitous globally. Recent studies are reporting microplastics found inside the gastrointestinal tracts of several marine species such as the semi-pelagic Boops boops. The ingestion of microplastics highlights the potential risk of heavy metals and persistent organic pollutants adhering to microplastic particles, potentially entering the food web affecting marine and human health. For the current study, the European anchovy (Engraulis encrasicolus) and the European pilchard (Sardina pilchardus) were sampled at several geographic locations in the Western Mediterranean Sea to determine the ingested abundance of microplastics in the gastrointestinal tract from both pelagic and benthic samples. Pelagic samples for both species were collected at an average depth of 111.67 m on the continental platform at of the Spanish Mediterranean Sea. Gastrointestinal tracts of 92 fish from 7 locations were examined with 14% of individuals found with ingested microplastics. To determine the chemical composition of the microplastic particles, Fourier Transform Infrared (FT-IR) is used to identify the polymer type. Results from this study will aid in determining the variation in microplastic ingestion between pelagic and benthic schools of the commercial fish species E. encrasicolus and S. pilchardus giving insight into the potential implications of microplastics in fish species for human consumption.

543 Microplastic ingestion in commercial and shark species in the Western Mediterranean Sea

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Samples of Mullus surmuletus and Galeus melastomus were obtained with commercial bottom trawling vessels in two areas of the Balearic Islands (western Mediterranean). Both species were seen to ingest microplastics, mainly blue filaments. However, analyses under the stereomicroscope and FTIR analyses revealed film type microplastics in gastrointestinal guts of G. melastomus and not in M. surmuletus. Microplastic ingestion ranged from 0.04 ± 0.04 to 0.92 ± 0.29 MPs/fish. According to M. surmuletus, highest percentage of affected individuals were given in the southwest area while for G. melastomus, percentage of affectation was highest in the northwest area. Variability of the results remark the need of sampling at multiple geographical scales and integrate different species in order to improve the approach to study micro-plastics in marine biota. In addition, chemical biomarkers were analysed for M. surmuletus, providing preliminary data on the response of antioxidant enzymes – catalase and superoxide dismutase, detoxification enzyme glutathione s-transferase (GST) and malondialdehyde (MDA) levels to microplastic ingestion.

Novel Mechanisms of Nanomaterial Toxicity Through Direct Exposure or Indirect Interactions with Environmental Components – Part 3

544 Elucidating the role of nonmaterial coating on uptake and translocation mechanisms at the sub-cellular level in plants using a hard X-ray nano probe

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The objective of this project was to understand the role of polymer coating charge on the uptake, translocation and subcellular distribution of CeO_2 in the tomato (Solanum lycopersicum cv Micro-Tom). Dextran (DEX; neutral), diethylaminoethyl dextran (DEAE; positive) and carboxymethyl detran (CM; negative)-coated CeO2NP (3-5 nm primary particle size) were synthesized and characterized for primary particle size (TEM), hydrodynamic diameter (DLS) and zeta potential (PALS) in exposure media. Tomato seedlings were grown in 10% Hoagland solution are exposed to DEAE, DEX and CM CeO_2NPs and control media for 2 weeks. The bulk tissue concentrations of Ce were determined in shoots, leaves alone and roots by inductively coupled plasma mass spectrometry (ICP-MS). Tissues were also collected from the root tip region and prepared by thin sectioning for Ce mapping using a synchrotron based X-ray fluorescence nanoprobe (nXRF) using the hard X-ray nanoprobe (HXN) at the National Synchrotron Light Source-II (Upton, NY, USA). The results indicate that CeO_2NP with positively charged coatings have the greatest impact on seedling growth. There is a trend of decreasing values of bioconcentration factor for all three CeO_2NP with increasing exposure concentration. Ce was concentrated mostly in the root tissue although there is significant translocation of Ce from the roots to shoots. The distribution of Ce in the root tip cells (down to 10-15 nm resolution) vary with the charge states of the coatings, with the apoplastic spaces showing the highest Ce accumulation concentrations, suggesting an apoplastic uptake mechanism. Entry into the root tip appears to occur in gaps between cells in the epidermis formed as cells are being shed from the root tip. Dissolution data showed that there was minimal Ce dissolution from the particles in the nutrient media used in the study. As the first general users of HXN, which is the highest resolution X-ray fluorescence microscope in existence, this study provides perhaps the highest resolution X-ray fluorescence imaging of nanomaterials in a biological tissue to date.

545 Nanoparticle Detection in Environmentally Relevant Matrices Using DMA-ICP-MS

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Engineered nanoparticles frequently are used as additives to consumer products to enhance product properties. Understanding the environmental fate and impact of engineered nanoparticles relies on the ability to characterize them at relatively low levels in complex media, which often contains additional naturally occurring nanoparticles. We present the application of a recently developed hyphenated technique combining electrospray aerosol generation, particle size selection using differential mobility analysis (DMA), and elemental analysis using inductively coupled plasma mass spectrometry (ICP-MS) to characterize gold and silver nanoparticles in environmentally relevant water samples including ground water, industrial wastewater, and ecotoxicology test media. The method is capable of detecting nanoparticles of 20-100 nm diameter at part-per-billion (ppb) levels in environmentally relevant aqueous solutions. Following instrument characterization, the DMA-ICP-MS was used to assess the fate of gold nanoparticles in a solution containing daphnia magna. Insights into dose verifications obtained by DMA-ICP-MS will be discussed as well as the method's performance and the advantages of the hyphenated DMA-ICP-MS over single-particle ICP-MS.

546 Development of Gold-labeled Titanium Dioxide Nanoparticles for Examining Exposure and Biodistribution in Complex Matrices

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Titanium dioxide nanoparticles (TiO_2 NPs) are produced in large quantities for multiple consumer and industrial applications, many of which will lead to the direct release of these materials into natural and engineered systems. Understanding the environmental effects of TiO_2 NPs is critical, but researchers must first overcome the challenge of distinguishing engineered NPs from high concentrations of naturally-occurring titanium. To better facilitate detection and quantification of TiO_2 NPs in complex systems, labeled particles with gold cores and TiO_2 shells were developed. The properties and behavior of the gold-labeled TiO_2 NPs were compared with those of unlabeled particles, with the goal of modifying synthetic procedures to minimize differences between the two NP types. The gold label is sensitive to a variety of analytical techniques useful for studying NP transport and behavior, including inductively coupled plasma mass spectrometry, hyperspectral imaging, and neutron activation analysis. The labeled NPs have been accurately quantified at parts-per-billion level concentrations in environmental samples containing background titanium. Ongoing experiments are designed to demonstrate the utility of the labeled particles in toxicity experiments by examining relevant properties like the potential to generate reactive oxygen species. The presence of a label that is easy to detect will allow for a wider range of experiments to be performed in environmentally-relevant exposure scenarios, including scenarios containing multiple organisms or nanoparticle types.

547 Does nanoclay technology adversely affect aquatic biota relative to natural nanoclays?

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Nanoclays represent a large class of manufactured nanomaterials (NMs) that have received great attention from the scientific and industrial communities. One important use of nanoclays is in wastewater treatment and pollution control as they can remove toxic chemicals from water supplies. Nevertheless, many concerns have been raised about the effect of manufactured nanomaterials on aquatic ecosystems as the nanomaterials themselves could have adverse effects. In this study, we investigated the potential toxic effects of a natural nanoclay (Na^+ montmorillonite) and two manufactured nanoclays (Cloisite®30B and Novaclay™) on Clamydomonas reinhardtii and Daphnia magna. Both freshwater specieswere exposed to a range of nanoclay concentrations to determine the NOAEL (No Observed Adverse Effect Level) and LOAEL (Lowest Observed Adverse Effect Level) of each type of nanoclay on the population growth of C. reinhardtii, and survivorship and growth rate of D. magna during acute (48h) and chronic tests (10 days). Our results indicated that an increase in nanoclay concentration negatively affected algal population growth but the effects manifested at a lower concentration for Cloisite® 30B (NOAEL: 1 mgL^{-1} and LOAEL: 10 mgL^{-1}) than it did for the other nanoclays (NOAEL: 1000 mgL^{-1} and LOAEL: 10000 mgL^{-1}). In addition, a very low concentration of Cloisite® 30B severely reduced the survivorship of Daphnia magna (NOAEL: 0.1 mgL^{-1} and LOAEL: 1 mgL^{-1}) for both acute and chronic tests. Novaclay™ also reduced daphnid survivorship at low concentrations (NOAEL: 0.1 mgL^{-1} and LOAEL: 1 mgL^{-1}) but only after chronic exposure. Natural nanoclays only reduced daphnid survival at higher concentrations (NOAEL: 10 mgL^{-1} and LOAEL: 100 mgL^{-1}) and only with acute exposure. We found little effect of natural nanoclays and Novaclay™ on the body growth of Daphnia and we were unable to assess the effects of Cloisite® 30B on the body growth of daphnids at higher concentrations, because all organisms died when exposed to Cloisite® 30B. Our work highlights that aquatic organisms are differentially susceptible to natural and manufactured nanoclays and most sensitive to Cloisite® 30B. Consequently, we should be careful about the kinds of manufactured nanoclays that we introduce into aquatic environments.

548 Differential uptake and toxicity of CuO nanoparticle to Daphnia magna from chronic exposure using two delivery scenarios

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Copper oxide nanoparticles (CuO NPs) are highly toxic to aquatic species, yet their extensive use in consumer products and industrial applications inevitably results in release into surface water. Currently, few studies have addressed whether CuO NPs can be transferred through the aquatic food chain. Here, we investigated the uptake and trophic transfer of CuO NPs from the algae Chlorella vulgaris to the crustacean Daphnia magna. We hypothesized that CuO NPs associated with algal cells could be transported to predators through feeding, and that the chronic toxicity resulting from this delivery scenario would differ from a direct CuO NP exposure. To test our hypothesis, D. magna were fed algae that had been incubated with CuO NPs or were directly exposed to CuO NPs while uptake and toxicity were evaluated. Strong surface associations and potential internalization of CuO NPs into algae were observed using hyperspectral imaging, and uptake was quantified with ICP-OES. We found high concentrations of Cu in D. magna bodies and molted carapaces in both exposure scenarios, suggesting that CuO NPs were taken up by D. magna and could be regulated through molting. We detected Cu in the neonates produced in the feeding exposure but not the direct exposure, implying that D. magna may metabolize CuO NPs differently depending on the method of delivery. Mean bioaccumulation factors were 12382 and 9899 in feeding and direct exposure scenarios, respectively, suggesting that CuO NPs had a high tendency to accumulate in D. manga bodies in both delivery scenarios. Significantly higher D. magna mortality was found in the direct exposure compared to the feeding exposure, which is likely due to decreased surface reactivity and dissolution caused by algae-particle hetero-aggregation as Cu^{2+} was undetectable. In addition, CuO NPs delivered through a feeding exposure significantly reduced neonate production compared to control. Thus, nanoparticle interaction with biota at one trophic level may alter the biological response to that nanoparticle at the next trophic level. This study highlights the importance of assessing nanomaterial ecological impacts in more environmentally relevant scenarios including multiple delivery scenarios and chronic sub-lethal exposures.

549 The release of "transparent blue" automobile coatings containing nanoscale copper phthalocyanine and their effects to aquatic organisms

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Nanoscale copper phthalocyanine (n-CuPc) has been increasingly used in different products, including printing inks, coating for automotive products, plastics, and textiles. Its use can improve the mechanical properties and add new features to the products, such as exhibit excellent transparency, lightfastness, heat stability, chemical and bleed resistance, processing capabilities and durability. It is estimated that Europe consumed an estimated 8 thousand metric tons of n-CuPc pigments (dry weight basis) in 2010. The automotive industry is the largest consumer of organic pigments, where increasing market requires stylish automobile coatings with vibrant colors "transparent blue" due to the pigments. This application of n-CuPc may result in release of n-CuPc release into the aquatic environment, especially during washing or repairing the automobile. Presently, very little is known about the exposure and hazard of n-CuPc in the aquatic environment, where n-CuPc may pose risks to freshwater and sediment organisms. The aim of the study is to develop methods and to generate data to assess the exposure and effects of n-CuPc in the aquatic environment. We estimate the release of n-CuPc from "transparent blue" automobile coatings through sanding approach, which is representative of a car repair process. Typically such repair operations are done in repair facilities where sanding dusts is collected and disposed, but an indirect emission from disposal into aquatic environment (e.g. freshwater) cannot be completely ruled out. Further, risk management measures (local exhaust on the sanding equipment, face masks) are typically in place at such operations to reduce occupational exposure. However, knowledge of the fate and hazard of sanding fragments in occupational settings is required. Accordingly, we investigated how the physicochemical properties of the released fragment n-CuPc from automobile coating changes in environmental (freshwater) and biological (cell culture) media and their potential effects to macrophages and aquatic environmental organisms.

550 Accumulation of Silver Nanoparticles in Aquatic Food Webs Following Pulsed vs. Repeated Exposure in Artificial Streams

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Given the rapid growth of the nanoparticle industry, the potential for nanoparticle discharge into the environment to incur ecological damage must be investigated. Silver nanoparticles (AgNPs) are commonly used in cleaning agents, clothing, and pharmaceuticals to eliminate unwanted bacterial growth. This diversity of applications increases the likelihood of AgNP entry into aquatic environments and subsequent exposure to organisms. Results from single species laboratory studies revealed that single pulse additions of < 100µg L^{-1}AgNPs cause mortality in algae, zooplankton, and gastropods, and accumulation of silver in sediment, periphyton, macroinvertebrates, and fish in lentic (wetland and pond) mesocosms. However, the fate of AgNPs in flowing water have not been examined lotic systems (i.e. streams) despite their potential to receive industrial and wastewater discharge as pulses or as repeated small amounts over time. We hypothesized that the fate of silver from small, frequent AgNP exposures differs from that following one-time large pulse exposure. We predicted that a large pulse should result in greater silver accumulation in sediments and bottom-dwelling stream inhabitants such as periphyton and C. decisum. In contrast, repeated smaller doses are more likely to maintain AgNPs in the water column and accumulate in Gambusia sp. due to increased exposure time. Artificial, recirculating streams were created by adding water from the Ogeechee River, GA, USA along with a simplified community consisting of sediment, primary producers (periphyton), a grazer (the snail C. decisum), and a consumer (the mosquitofish Gambusia sp.). The stream communities were exposed to one of five treatments for two-weeks: no-addition control, AgNP doses of 5µg L^{-1} every two days, AgNP doses of 10µg L^{-1} every two days, a one-time addition of 35µg L^{-1}, and a one-time addition of 70µg L^{-1}. Water samples were analyzed for Ag content initially, and one and two weeks post-exposure to treatments. Sediment, periphyton, C. decisum, and Gambusia sp. were analyzed initially and at the end of the experiment to determine AgNP accumulation. Ag content was quantified using inductively coupled plasma-mass spectrometry (ICP-MS). Results from this study will provide valuable information to assist in determining the need for additional monitoring and regulation of AgNPs entering the environment.

551 Multigenerational Effects of Silver Nanomaterials in Caenorhabditis elegans

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Studies of the effects of as-synthesized and transformed sulfidized silver nanomaterials (Ag-NMs) to a model organism, a nematode Caenorhabditis elegans have shown increase in toxicity as well as unique transcriptomic responses after single-generation exposure. Recent multigenerational study has also shown enhanced sensitivity for reproductive toxicity over multiple generations. However, genomic effects (e.g. mutations and epimutations) that can be induced by exposing C. elegans continuously to nanomaterials over multiple generations have not yet been investigated. In this study, we used wild type and DNA mismatch repair mutant (msh-2) strains of C. elegans to determine if mutations resulting from Ag-NM and ion exposures can contribute to the observed multigenerational effects. Exposures to both as synthesized and sulfidized Ag-NMs as well as their respective ions were conducted for 10 generations using sub-lethal concentrations of EC_{30} for reproduction. Whole genome DNA sequencing approach using NextSeq was applied to a wild-type C. elegans to examine and compare mutations before and after multigenerational exposures within and among treatments between F_0 and F_{10} generations. In addition, individual 72-96 nematodes from N2 and msh-2 strains from F_0 and F_5 generations were collected for microsatellite instability analysis. The sequencing data have been aligned to the reference genome and comparisons within (from F0 to F10) and among treatments are being made to test whether exposure to Ag-NPs can induce mutations and result in germline instability. Our preliminary results suggest that exposure to as-synthesized Ag-NMs resulted in increase in missense, deletion and insertion mutations and these mutations were observed in coding, regulatory 5' and 3' untranslated regions, intergenic regions, and introns. For microsatellite analysis, there were no mutations detected at the four analyzed microsatellite loci for the wild type nematodes. However, mutations were detected for the mismatch repair deficient msh-2 mutant at one locus with mutation rate slightly increasing over four generations from 1.04 x 10^{-2} in controlsto 4.17x 10^{-2} after exposure to as synthesized Ag-NPs. The nature of mutations differed between control (deletions) and exposed (insertions) treatments. Analyses of the sequencing data and more microsatellite loci are ongoing to determine if exposure to Ag-NMs can lead to accumulation of mutations resulting in overall genomic instability.

Fate and Effects of Metals in the Environment: Modeling and Interpreting Effects of Metals Mixtures

552 Internal versus external dose for describing ternary metal mixture (Ni, Cu, Cd) chronic toxicity to Lemna minor

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Resolving the inability to predict risk from metal mixtures in waters surrounding Canada's many current and legacy extractive mining sites is a high priority for the government and base metal mining companies. Mixtures of metals may have toxicity that would not be predicted additively, as uptake of metals into an aquatic organism is influenced by water chemistry factors, which in turn impacts bioavailability (i.e. interaction of the metal with the biological receptor called the biotic ligand or BL). Our study aimed to validate the 'concentration addition' (CA) approach to predict the toxicity of a ternary metal mixture (Ni, Cu, Cd) to Lemna minor (one of Environment Canada's recommended test plant species for bio-monitoring of mining effluents). Simultaneous determination of total metal concentration and free metal ion activity in solution (external dose) and metal accumulation in plants (internal dose) was done to assess the best representative of 'bioavailable dose' for use with the CA approach. In addition, a mechanistic investigation using accumulation kinetics and response surface regression of internal dose versus root growth inhibition was conducted. The results of this indicate that Ni-Cu-Cd likely compete with each other for uptake into the plant, but once inside, Cu-Cd share binding site while Ni does not. A synergistic interaction between Cu-Cd was suggested by the increase in binding affinity (Kd) from single metal to mixture exposure for the two metals (Ni had the opposite effect). This study also demonstrates that concentration addition is an appropriate model for estimating mixture toxicity when dose metric approximates amount of metal delivered to plant (internal dose), suggesting that risk assessments for metals in water should utilize internal tissue metal concentration as an indicator of dose.

553 The unexpected effects of metal mixtures on zebrafish gills

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It is challenging to predict the effects of metal mixtures. When in combination they not necessarily follow the trends of effects presented during single metal exposures, the effects can be more than additive, similar or even antagonistic. In order to expand the knowledge on the effects of metal mixtures in fish we evaluated the influence of isolated or binary combinations of metals on unidirectional (measured with ^{22}Na) and net sodium fluxes and immunolabeling of gill ion transporters and metallothionein in zebrafish (Danio rerio). In order to challenge fish with comparable toxicity levels we exposed them to 10% of their LC50 in single and binary tests with copper (Cu), nickel (Ni) and cadmium (Cd). The concentrations used were 6.2 ug/L of copper, 280 ug/L of cadmium and 1200 ug/L of nickel during 3 and 96h exposures in static and semi-static systems, respectively. The 3h net ion fluxes were significantly different from 96h fluxes in fish exposed to Cu and Cd; in both cases they switched from a net gain to a net loss of sodium. The common response observed in other fish species is to present sodium net loss after exposure to metals as copper. Indeed, zebrafish seems to respond differently in this regard, presenting net sodium gain when exposed to single Cu and Cd, while the cadmium combinations Cu+Cd and Ni+Cd induced the opposite effect, with the fish presenting significant sodium losses after 96h of exposure. The indirect immunofluorescent localization of metallothionein was increased in all treatments. Interestingly the metallothionein was not labeled exclusively in mitochondria rich cells in zebrafish as usually observed in other fish species. We observed that metals can modulate the immunolabelling of ion transporters such as vH-ATPase and Na,K,ATPase on zebrafish gills. Our findings indicate that the very short-term response (3h) cannot be used to predict a longer term response (96h), and also that single metal effects are not good predictors of combined metal effects (CNPq, NSERC Discovery, IDRC).

554 Effects of binary mixtures of Ag, Cd, Cu, Ni, Pb and Zn to the freshwater snail Lymnaea stagnalis – short term uptake and chronic toxicity

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Incorporating metal mixture toxicity into regulatory frameworks has been an important focus of environmental scientists and regulators over the past decade. As a result, great progress has been made in the development of new experimental, statistical and modeling approaches for the interpretation of metal mixture toxicity. Nevertheless, more data are needed to continue improving our understanding on this complex subject. Specifically, the chronic toxicity of metal mixtures have been sparsely investigated in contrast to acute toxicity, despite its generally greater relevance for environmental risk assessment. The freshwater snail Lymnaea stagnalis is chronically highly sensitive to a range of trace metals. In the present study, metal interactions were assessed in juvenile L. stagnalis exposed to binary mixtures of six environmentally relevant metals (Ag, Cd, Cu, Ni, Pb and Zn), with 14-d growth, 2-h metal uptake and tissue distribution experiments. Results so far indicate that metals do not strongly interact with each other in both growth and short-term uptake tests. [This work is funded with a CRD grant from NSERC, in partnership with Rio Tinto, ICA, CDA, NiPERA and IZA].

555 The toxicity of mixtures of selenium, cadmium, nitrate, and sulphate to Ceriodaphnia dubia

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The potential for unexpected interactions among constituents to result in increased toxicity has been identified by the scientific community and regulatory bodies as a source of uncertainty in the development of water quality guidelines and objectives that are protective of aquatic life. The purpose of this research was to assess the potential for the toxicity of mixtures of selenium (Se), cadmium (Cd), nitrate (NO3) and sulphate (SO4) to differ from the toxicity of individual constituents. Test solutions were evaluated with a Ceriodaphnia dubia 7-d reproduction and survival test according to Environment Canada procedures. Eleven mixture toxicity tests were conducted with constituent concentrations ranging from 0.02 to 1 µg Cd/L, 3.7 to 52.7 mg NO3-N/L, 19.5 to 139 µg Se /L and 117 to 934 mg SO4/L in laboratory water or water from a site in British Columbia, Canada. The sensitivity of C. dubia to NO3 and SO4 was also assessed by conducting constituent-specific toxicity tests in a range of water types. Survival and reproduction effects were observed at higher mixture concentrations and were attributed to NO3 and SO4.

556 X-ray fluorescence based examination of zinc distribution and speciation in rainbow trout gills: interactions with copper or cadmium

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The current framework of the biotic ligand model (BLM) is based on the metal binding to the fish gills. Although the BLM approach has been successfully used to predict the toxicity of single metals in aquatic organisms, its potential to evaluate the metal mixture toxicity is presently under investigation. Understanding metal interactions in the fish gills would be a key step in the process of developing a sound metal mixture BLM. To this end, we employed X-ray fluorescence imaging (XFI) and micro X-ray absorption near-edge spectroscopy (XANES), which are both synchrotron based techniques, to examine: (i) the spatial distribution and chemical speciation of Zn and its co-localization pattern with other essential elements (Ca, S and Fe), and (ii) the effect of competing metals (Cd and Cu) on the Zn distribution and speciation in fish gills. Ranbow trout (150-200g) were exposed to acute (96-h LC-50) levels of waterborne Zn, singly and in combination with Cu or Cd, for 24-h. Following exposure, gills were dissected out, and 5 micron thick sections were prepared to anylyze Zn distribution and speciation profiles in the rainbow trout gills using the multi-element hard X-ray microprobe at the VESPER beamline, Canadian Light Source, Saskatoon, Canada. Zinc was found to accumulate mainly in the primary lamellae of the gill, which corresponded to the high density of chloride cell localization, supporting the putative roles of these cells in branchial metal uptake. In addition, Zn was found to predominantly co-localize with Ca and S, but not with Fe, indicating that the Ca- and S- containing moieties are involved in intracellular Zn handling. The spatial distribution of Zn in the gills was markedly reduced during co-exposure to Cd, but not to Cu, suggesting a competitive interaction between Zn and Cd for branchial uptake. Moreover, the predominant chemical species of Zn in the trout gills were Zn-phosphate, Zn-oxide and Zn-cysteine. Co-exposure to Zn and Cu did not alter the chemical speciation of Zn, however a notable increase in the fraction of Zn-cysteine was recorded during co-exposure to Zn and Cd, possibly due to the metallothionein induction (increased detoxification capacity) in fish. To the best of our knowledge, this is the first study to examine how metal mixture interactions influence the spatial distribution and chamical speciation of metals in the fish gills, and our findings have important implications for understanding metal mixture toxicity in fish.

557 Interaction and toxicity of cadmium, copper, and nickel on the olfactory system of rainbow trout Oncorhynchus mykiss Walbaum, 1792

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Water quality criteria are mainly based on the effects of individual contaminants, even though numerous studies have demonstrated that interactions among contaminants may affect the overall toxicity. In the present study the toxic effects and interactions of nickel (Ni), cadmium (Cd), and copper (Cu) on the olfactory response of rainbow trout were determined. To study the toxic effect of single metals on the olfactory system, rainbow trout were exposed to a geometric dilution series of each metal for 24 h. Olfactory acuity was tested using electro-olfactography (EOG). Based on the inhibitory concentration (IC) curves the 20% inhibitory concentration (IC20) of each metal was calculated. Fish were exposed to binary and ternary mixtures of the three metals at the determined IC20 for each metal for 24 h and the acuity of the olfactory system was measured using EOG. In order to find out whether or not rainbow trout can detect the metals using olfaction, fish were tested for their EOG response to 10^{-6} M and water quality criterion concentrations of each metal. The IC20 of Cd and Cu on olfactory function of rainbow trout was calculated at 24.4 and 4.6 µg/L (2.2×10^{-7} and 7.2×10^{-8} M), respectively. Nickel did not impair rainbow trout olfactory function at environmentally relevant concentrations, therefore, in the mixture experiment the acute water quality criterion for Ni 770 µg/L (13.6×10^{-6} M) was used. Binary mixtures of Ni + Cd and Ni + Cu showed synergic olfactory impairment, while Cd + Cu showed an antagonistic olfactory impairment. Rainbow trout were able to detect all three metals at both 10^{-6} M and water quality criteria concentrations. The results of the current study suggest that some of the criteria used for the protection of aquatic life, such as the Cu acute criterion, might not be protective against adverse effects of even single contaminants on the olfactory system of fish. Additionally, interactions between some specific contaminant mixtures, such as Ni with Cd or Cu, might increase the toxicity of each individual contaminant. Current water quality criteria do not take these mixture interactions into account. On the other hand, rainbow trout may be able to detect and probably avoid contaminant metals at criteria concentrations.

558 Metal-mixture toxicity (Copper + Nickel + Zinc) to aquatic insect communities in mesocosms

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Surface waters are nearly always comprised of mixtures of metals while aquatic life criterions are applied one metal at a time. There is virtually no data on metal mixture effects on aquatic insect communities, thus it is generally unknown if metal-mixtures are more or less toxic than expected from the sum of effects of metals individually (response addition). We have conducted four, 30-day mesocosm evaluations of metal-mixtures with the goal of developing a predictive model of the effects of metal-mixtures on aquatic ecosystems. Having executed experiments using Copper (Cu), Cadmium (Cd), and Zinc (Zn) singularly and in mixture, here we present early results of a Cu, Nickel (Ni), and Zn experiment. Nominal aqueous dissolved concentrations ranged from 1 – 100 µg/L for Cu, 0.3 – 800 µg/L for Ni, and 1 – 1600 µg/L for Zn. Average water quality characteristics were 15 mg/L alkalinity as $CaCO_3$, 16.6 mg/L hardness as $CaCO_3$, and 3.2 mg/L dissolved organic carbon. Toxicity to mayfly abundance was greater for Cu (LC50 = 16.6 µg/L nominal) then for Ni (53.62 µg/L nominal) or Zn (198.5 µg/L nominal). LC50 values are expected to fall once measured concentrations are available because observed concentrations in mesocosms are regularly 30-60% lower than nominals. In all cases, given the assumptions of response addition, Ni + Zn mixtures were less toxic than expected, as was true for Cu + Ni + Zn. Prior mesocosm tests also support this finding; generally metal-mixtures are less toxic than expected but not grossly different from additivity. Thus, the assumption of additivity commonly used in risk assessments of the effects of metal-mixtures on aquatic insect communities continues to be a reasonable assumption.

559 Modeling the Chronic Effects of Metal Mixtures to Aquatic Organisms: A Meta-analysis

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Although metals in the aquatic environment mostly occur as mixtures, ecological risk assessment procedures are currently based on a metal-by-metal approach. Recently, different methods to evaluate risks of metal mixture exposures have been proposed. These methods combine the bioavailability-based SSD-approaches of the individual metals with two generally used mixture reference models, concentration addition (CA) and independent action (IA). However, the assumptions underlying each of these methods have not yet been tested. In several experiments conducted over the past years, we investigated chronic toxicity of Ni, Zn, Pb, Cu and Cd mixtures in different combinations to the crustacean Ceriodaphnia dubia (7d reproduction), Daphnia magna (21d reproduction) and the algae

Pseudokirchneriella subcapitata (72h growth). In the present study, we combined all our mixture toxicity data in a meta-analysis to evaluate two risk assessment related questions at the single species-level: I) Which of the two commonly applied mixture reference models (CA or IA) describe metal mixture toxicity most accurately; II) Which of those two models is most conservative for chronic mixture toxicity? For C. dubia, the IA model described metal mixture toxicity clearly more accurately than the CA model. However, for D. magna and P. subcapitata the CA model performed slightly better than the IA model. In general, the CA model was the most conservative model. In addition, we observed that the CA model was generally protective at the effect sizes relevant for environmental risk assessment frameworks, but also overestimated metal mixture toxicity at the EC10 level on average by 1.4 fold. Finally, we compared the prediction performance of two metal mixture bioavailability models (MMBM), an IA based MMBM and a CA based MMBM (WHAM-F_{Tox}) for predicting chronic Ni-Zn-Pb mixture toxicity to C. dubia. The IA MMBM predicted metal mixture toxicity more accurately than the WHAM-F_{Tox} model. In addition, the MMBM had a higher predictive potential than an IA model simply relying on free metal activity or on the dissolved metal concentration. Overall, our study confirms that the CA reference model can be used as a conservative first tier in a tiered metal mixture risk evaluation scheme, but more accurate models are needed in higher tiers. Calibrated metal mixture bioavailability models can predict mixture toxicity more accurately than models merely based on dissolved concentrations or free ion activities.

Developments and Barriers in the Adoption of Amendments for Soil and Sediment Remediation

560 Tools to overcome barriers for in-situ sediment treatment in the U.S

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Extensive experimental studies and field trials have shown that, when applied correctly, in-situ treatment via contaminant sequestration and immobilization using a sorbent material such as activated carbon has now progressed from an innovative sediment remediation approach to a proven, reliable technology. However, there are still significant institutional barriers that limit its application. This presentation will summarize key tools that have proven effective in advancing this technology and getting in-situ treatment incorporated into the remedy at a site. First, an important initial step is to develop a robust conceptual site model that presents a clear understanding of sediment dynamics and stability, and ongoing sources and background levels of contamination. This step is essential for gaining regulatory and stakeholder acceptance of sites for which in-situ treatment can be effective. Second, comparative evaluations of remedial alternatives, building on laboratory and field studies and using modeling as appropriate, are needed to accurately contrast the short- and long-term impacts and effectiveness of in-situ treatment versus more traditional remediation methods such as dredging and capping or natural recovery processes. This allows for proper site-specific balancing of the potential benefits, risks, ecological effects, and costs of in-situ treatment relative to other sediment cleanup technologies. In particular, the type and size of amendments can have effects on persistence, kinetics driving bioavailability, and ecological effects, often defining clear tradeoffs that have differing importance with key stakeholders and resource and regulatory agencies. Third, while there are few remaining technical issues associated with in-situ treatment that have not already been thoroughly addressed in previous field trials and full-scale applications, performing pilot studies focused on specific agency or stakeholder concerns can be pivotal to achieving acceptance. Such trials are important because contaminated sediment sites often have unique physical, chemical, and hydrodynamic factors affecting remedial design. Finally, early identification and focus on cost-effective designs and application methods that appropriately address site-specific conditions can streamline the overall evaluation. Lessons learned from completed and ongoing in-situ treatment projects will be reviewed.

561 Modeling Activated Carbon Amendments in Shallow Ecosystems

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In the past decade significant advancements have been made to understand the effectiveness and ecological benefits of amending activated carbon (AC) to sediments contaminated with hydrophobic organic compounds (HOCs). Laboratory as well as pilot-scale field studies have shown that application of up to 4% AC results in effective removal of sediment porewater HOC and consecutively reduced bioaccumulation in benthic invertebrates and fish [1,2]. Therefore, amendment strategies involving AC are rapidly gaining popularity and viewed as a sediment management option. However, integrative models of the fate and transfer of HOCs in AC amended ecological systems in situ are scarce. Current biouptake models may not adequately explain responses to different AC treatments in natural ecosystems with varying food availability and thus predict long-term bioaccumulation of HOCs in aquatic organisms. In this study, we developed an integrated mass balancing approach to model fate and transfer of polychlorinated biphenyls (PCBs) in ditch ecosystems amended with powdered (PAC), granular AC (GAC) and with subsequent removal of AC granules, i.e. sediment stripping to interpret the bioaccumulation results in our test systems. The PCB concentrations in the aqueous phase was modeled using multiple terms for exchange between (a) porewater, (b) overlying water, (c) air (d) fish, (e) macrophytes, (f) zooplankton, (g) invertebrates, (h) slow sediment sorption domains, (i) PAC or GAC and (j) degradation. This presentation will focus on the development and application of the ecosystem model and discuss the application of the model for AC amended systems. Specifically, the agreement of the model with observed PCB body burdens for fish, macrophytes and zooplankton with and without sorbent amendment will be discussed. The model integrates across the various phases and fauna and provides a more realistic assessment of the ecological response to AC in field settings.

562 Activated carbon amendments in PCB contaminated sediment: full life cycle test with Chironomus riparius

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Carbon amendments have been shown to be an effective in-situ remediation method for contaminated sediments. Especially activated carbon (AC) amendments have a strong sorption capacity towards many hydrophobic organic compounds, and they have been shown to reduce aqueous and bioaccumulated concentrations both in laboratory and field studies. Along with the remedial benefits, direct adverse effects of these amendments have been reported, but the mechanism behind the adverse effects is still unknown. In addition, the effects of amendments on transport of contaminants directly from aquatic to terrestrial ecosystems by metamorphic species has not been considered before. The objectives of this study were to examine the remediation potential and possible secondary effects of AC on Chironomus riparius in a full life cycle test. AC (ø 63−200 µm) mixed into the sediment efficiently reduced PCB bioavailability determined by C. riparius bioaccumulation tests and passive samplers. Additionally, the PCB concentrations in midges emerging from AC treated sediments were reduced. Slightly improved reproduction, survival, larvae growth and gut wall microvilli length was observed with low AC (0.5% sediment dw) dose in the sediment with low organic carbon content. However, higher AC doses (2.5% sediment dw) caused adverse effects on emergence and larval development in both study sediments. In addition, morphological changes in the gut wall microvilli layer were observed. Metamorphic species, such as C. riparius, may act as a vector for organic pollutants from aquatic to terrestrial ecosystems and according to this study AC amendments may reduce this transport. The fundamental question of balancing between the effective remedial efficiency and possible secondary effects is central in every remedial action designed. With carefully selected AC dosages and by knowing the sediment characteristics the degree of the adverse effects may be reduced. The carbon amendment method is promising, but further research is still needed to thoroughly understand the mechanism behind the secondary effects.

563 Remediation of PCB contaminated sediments using activated carbon: Thermodynamic exposure assessment

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The overall aim of this project was to reveal how activated carbon (AC) amendments can be most effectively applied to (1) reduce exposure of polychlorinated biphenyls (PCBs) in sediment and (2) enhance resilience of in situ sediment remedies challenged by ongoing influx of contamination. For this purpose, exposure assessments were conducted in a series of mesocosms experiments: Nereis virens (worm), Mercenaria mercenaria (clam) and Cyprinodon variegatus (fish) were exposed to PCB contaminated sediment (New Bedford Harbour, MA, USA) that was (i) amended with AC, (ii) covered by a sand cap, (iii) covered by an AC amended sand cap and (iv) covered by a mobile AC amended sand cap. Half of the mesocosms received an ongoing influx of contamination during the 90-d experiments. Quantitative thermodynamic exposure assessment (Q-TEA) was conducted before and after AC treatment based on in situ passive sampling using polyethylene discs/strips and ex situ equilibrium sampling using jars coated with µm-thin layers of silicone. Up to 130 PCB congeners were measured by each method. Based on equilibrium concentrations in polymers, activity ratios between treatments (i-iv) were determined in order to assess exposure reduction efficiency, whereas resilience of remedies was assessed from activity ratios between treatments receiving ongoing non-spiked and PCB-spiked sediment. From equilibrium sampling results, PCB sorption by AC was found to depend on the level of chlorination and ranged from 37% exposure reduction of hepta-chlorinated PCBs to 99% exposure reduction of tri-chlorinated PCBs. These results were supported by measurements in worms, clams and fish. Then, polymer concentrations were translated into freely dissolved PCB concentrations in sediment pore water (C_{free}, ng/L), and results based on in situ passive sampling and ex situ equilibrium sampling cross validated each other. Further, passive sampling was used to determine C_{free} in surface water, and these results were applied to determine the equilibrium status between sediment and water. Finally, equilibrium sampling was used to determine PCB concentrations in lipid at thermodynamic equilibrium with sediment ($C_{lipid \rightleftharpoons sediment}$, mmol/kg) that was compared to lipid normalised concentrations in biota in order to determine the equilibrium status between sediment and organisms. Further results of Q-TEA and tissue analyses will be used to assess how application impacts in situ AC treatment effectiveness.

564 How much is enough? What more is needed to gain acceptance on the use of amendments as a safe and viable treatment technology

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Sorbent amendments are well-established treatments of organic chemicals in water supplies and groundwater. Increasingly, they are being tested on metals in surface water, sediment, and soils. Approval to remediate these media in situ with sorbent amendments requires demonstration of effectiveness and evaluation of potential adverse ecological effects. A suite of such studies is focused on Biochar treatment of mercury (Hg) in surface water, sediment, and soils at a Hg impacted site on the South River, Virginia. Test methodologies included bench top, mesocosm, and pilot-scale field studies. Effectivenesswas measured as reduced concentrations of bioavailable mercury and reduced bioaccumulation in lower trophic level organisms. Geochemical analyses generally confirmed that biochar effectively reduced concentrations of Hg and methylmercury (MeHg) in, soil, pore water, and surface water from the South River. Additionally, Biochar reduced Hg methylation rates in pore water. Bioaccumulation was reduced in multiple biological end-points that were evaluated including algae, aquatic and terrestrial invertebrates, amphibians and young-of-year fish. Unintended consequences of biochar were measured as potential adverse effects on lower trophic level organisms and plants in laboratory studies. Biochar produced little or no effect on detrital processing, and plant and earthworm survival/growth in lab studies. Although studies of lotic macroinvertebrate communities suggest the potential for effects of fine-grained biochar (but not larger sizes) on community metabolism, no effects were observed for community composition in a treated pond. Further studies on benthic community effects and Hg reduction in floodplain soils and earthworms are on-going. In aggregate, these studies demonstrate that biochar effectively reduces Hg and MeHg bioavailability in this system, and suggest limited (or no) adverse effects.

565 Ongoing Degradation of an Organophilic Clay Amendment in a Sediment Cap 11 Years after Placement

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A one foot thick layer of granular organophilic clay (Aqua Technologies ET-1) (OC) was placed within the McCormick & Baxter sediment cap in Portland, Oregon to reduce the potential of creosote seeps from reaching the river. Immediately after its emplacement in summer 2004, ebullition was observed overtop the footprint of the OC placement at significantly higher rates than what was observed in other locations within the sediment cap or elsewhere in the river adjacent to the sediment cap. In order to understand the ebullition, extensive study was conducted on the OC from the sediment cap. No creosote observed in any of the OC cores from the site, and it was assumed that due to the installation of a barrier wall, the source to potential creosote seeps had been eliminated by the wall. Organic matter and PAHs were analyzed from the ET-1 in 2008, 2009 and 2015. Low level PAHs were detected within the OC that are consistent with low level PAHs present in groundwater passing through the sediment cap. Low-level PAHs were detected at concentrations that typically fell between the method detection limit and reported detection limit. The summation of carcinogenic PAHs range from 0.03 mg/kg to 0.4591 mg/kg. In a 2008 study, the average organic matter content of several site OC samples was 15.9 percent (+/- 2.6 percent). Fresh OC organic content has an average of 24.1 percent (+/- 0.16 percent). At that time, the OC had been in place within the sediment cap for 4 years, which results in an estimated half-life for the organic matter within the clay of 6.6 years. Since the PAH concentrations within the OC are very low and there are no other significant sources of organic carbon expected to sorb to the OC within the sediment cap, the primary source of organic matter measured in the OC is within the structure of the OC itself. In 2015, the organic content ranged from 7.78 to 9.85 percent with an average of 8.56 percent. These results indicate that the OC is continuing to degrade with a half-life on the order of approximately 6 to 7 years. If the clay continues to degrade at this rate, it will return to its original bentonite form in approximately 40 years after installation of the sediment cap. While the degradation of the OC has not resulted in protectiveness issues at the Site, it has likely caused the limited buckling of the sediment cap observable in areas where the OC was placed due to loss of mass.

566 Predicting Cu and Zn sorption capacity of biochars based on source material and pyrolysis temperature

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Biochars are produced by combusting organic materials in the absence of oxygen (pyrolysis). There has been recent interest in using biochars to remediate metal contaminated soils and sediments due to their low cost, high surface area, resistance to degradation and high sorption capacity for metals. The diversity of biochars is huge because they can be produced from almost any organic material under a range of conditions (e.g. pyrolysis temperature, heating rate, and duration, or surrounding gases). However, a mechanistic understanding of how source materials and pyrolysis conditions contribute to the metal sorption capacity of the resulting biochar is lacking. This lack of understanding prevents us from predicting the performance of a biochar, and thus prevents us from producing custom biochars under defined conditions for specific applications, without experimentation. We produced biochars form 10 different organic materials by pyrolysing at 450°C. We then produced a further 10 biochars from one organic material (cedar wood) by pyrolysing at 10 different temperatures, increasing at 50°C intervals from 250°C to 700°C. Batch sorption experiments were conducted on all 20 biochar samples to construct Cu and Zn sorption isotherms. Isotherms were fitted to a Langmuir sorption model to derive the maximum Cu and Zn sorption capacity of each biochar. Cu and Zn sorption capacity of 450°C biochars could be predicted from the C:N ratio of the source material prior to pyrolysis. We also found a positive sigmoidal relationship between the pyrolysis temperature of the cedar wood biochar and the maximum Cu and Zn sorption capacity. FTIR analysis of the biochars revealed that as pyrolysis temperature increased there was a reduction in the abundance of oxygenated functional groups on the surface of the biochar and an increase in aromaticity. We therefore conclude that the higher sorption capacity of biochar produced at a higher temperature is due to an electrostatic attraction between the positively charged Cu and Zn ions and the delocalised pi-electrons on aromatic structures on the electronegative surface of the biochar. These relationships between biochar production parameters (source material and pyrolysis temperature) and the metal sorption capacity of the resulting biochar are the first step towards creating a biochar sorption model that will enable us to predict and produce custom biochars for specific applications, without costly experimentation.

567 Contaminant remediation: lessons learned from an ongoing field experiment with biochar to remediate a petroleum contaminated soil in Havana, Cuba

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Our recent monitoring campaign to determine organic contaminants in soils of the Havana province in Cuba revealed elevated (>10 mg/kg) polycyclic aromatic hydrocarbon (PAHs) concentrations at some sites. An area close to Havana bay contained the highest concentrations (40 mg/kg for the sum of the 16 USEPA PAHs). At this site soil is used to clean empty petroleum tanks. The petroleum-soil mixture is added to natural soil, homogenized again, and disposed on a field of 7.2 ha. The petroleum soil layer is weekly ploughed to air the soil surface layer and thus stimulate petroleum degrading microorganisms. After about a year of natural biodegradation, total hydrocarbons, fats and oils are analysed. Only when these contaminants are below a certain threshold value, the soil will be re-used to clean petroleum tanks. However, there is no data and therefore quality control about the fate of PAHs in the contaminated soil. Currently, we are about to establish a field experiment at this location. Its overall aim is to (i) shed light on the PAH biodegradation potential of the current remediation procedure, and (ii) to evaluate whether biochar amendment leads to a PAH exposure reduction. The field experiment includes 3 plots of 5 m x 5 m that serve as control and 3 plots where 5% biochar will be added to a fresh petroleum-soil layer. Biochar is locally produced from wood logs in a traditional kiln, and will be milled before use. This setup is repeated at a site of the field where bioremediation had been going on for almost a year. To quantify the freely dissolved concentration of PAHs in the pore water low density polyethylene strips will be deployed in the soil. The strips are loaded with PAH-like performance reference compounds to account for sorption kinetics. Current barriers of our activities are manifold and encompass, among others, the following aspects: i) limited awareness and/or prioritization of environmental risks by the different stakeholders mostly due to the lack of legislation and ii) technical and infrastructural limitations in Cuba, and iii) limited knowledge of new remediation approaches such as biochar amendment.

Strategies for Assessing Chemicals for Endocrine Activity: Making the Best Use of Screening-Level Information

568 Harnessing High-Throughput Monitoring Methods to Strengthen 21st Century Risk-Based Evaluations

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Over the past ten years, the US government has invested in high-throughput (HT) methods to screen chemicals for biological activity. Under the interagency Tox21 consortium and the US Environmental Protection Agency's (EPA) ToxCast™ program, thousands of chemicals have been evaluated for bioactivity across hundreds of assays. While these efforts support hazard-based prioritizations, exposure-focused programs have also emerged (e.g., EPA's ExpoCast™ program) to translate hazard data into risk- based decisions. This integrated screening framework offers an unprecedented means to improve chemical safety assessments in the US. Despite their obvious benefits, HT screening programs are not without challenges. The ToxCast program faces the daunting task of measuring bioactivity for thousands of registered chemicals, while attempting to model activity for unknown numbers of potential biological metabolites and environmental transformation products – data are needed to help prioritize the larger universe of candidate chemicals for further testing. The ExpoCast program is developing efficient models to estimate potential exposures, but lacks measurement data needed for parameterization and evaluation. This lack of measurement data, in both environmental and biological systems, often leads to large uncertainties in final exposure estimates. Clearly, HT chemical monitoring methods are needed to support ongoing efforts in the ToxCast and ExpoCast programs. Suspect screening and non- targeted analysis methods (SSA and NTA, respectively) are well suited to meet these needs, as they can rapidly generate measurement data for thousands of previously unstudied chemicals. Noting the opportunity to fill critical knowledge gaps, efforts are underway at EPA to develop, evaluate, and effectively use SSA and NTA workflows and data. As a reflection of these efforts, this presentation will highlight: 1) a framework for integrating SSA/NTA research within EPA's ToxCast and ExpoCast programs; 2) results of a SSA case study that exploited ToxCast and ExpoCast data to identify emerging contaminants in house dust; and 3) software tools and databases being developed within EPA to support SSA/NTA activities across the broader research community. The goals of this presentation are to communicate EPA's ongoing SSA/NTA research efforts, and to foster discussion on the potential contributions of HT monitoring methods towards advancing 21st century risk-based evaluations.

569 Application of ToxCast High-Throughput Screening and Modeling Approaches to Identify Steroidogenesis Disruptors

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Environmental chemicals can elicit endocrine disruption by altering steroid hormone biosynthesis and metabolism (steroidogenesis) causing adverse reproductive and developmental effects across mammalian and aquatic species. Historically, a lack of assays resulted in few chemicals having been evaluated for effects on steroidogenesis. The steroidogenic pathway is a series of hydroxylation and dehydrogenation steps carried out by CYP450 and hydroxysteroid dehydrogenase enzymes, yet the only enzyme in the pathway for which a high-throughput screening (HTS) assay has been developed is aromatase (CYP19A1), responsible for the aromatization of androgens to estrogens. Recently, the ToxCast HTS program adapted the OECD validated H295R steroidogenesis assay using human adrenocortical carcinoma cells into a high-throughput model to quantitatively assess the concentration-dependent (0.003-100 µM) effects of chemicals on 10 steroid hormones including progestagens, androgens, estrogens and glucocorticoids. These results, in combination with two CYP19A1 inhibition assays, comprise a large dataset amenable to clustering approaches supporting the identification and characterization of putative mechanisms of action (pMOA) for steroidogenesis disruption. In total, 514 chemicals were tested in all CYP19A1 and steroidogenesis assays. Of these, 216 chemicals were identified as CYP19A1 inhibitors in at least one CYP19A1 assay, and 208 of these chemicals also altered hormone levels in the H295R assay, suggesting 96% sensitivity in the ability of hormone quantification to identify CYP19A1 inhibitors. The steroidogenesis data were also used to model CYP17A1 inhibition. Using increases in progestagens and glucocorticoids with simultaneous decreases in androgens and estrogens as a profile indicative of CYP17A1 inhibition, known CYP17A1 inhibitors such as conazole fungicides including prochloraz were correctly identified. Cumulatively, our examples demonstrate that computational profiling of in vitro data provides a model that characterizes pMOAs for steroidogenesis disruption to support the identification of endocrine disruptive chemicals. This abstract does not necessarily reflect USEPA policy.

570 The developing fathead minnow as a screen for thyroid disrupting compounds: Identification of sensitive endpoints

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The fathead minnow (Pimephales promelas) is often used as a model organism to assess acute and chronic toxicity and to screen for reproductive endocrine disrupting compounds. In recent years, developing fathead minnows have also been used to evaluate the impacts of environmental contaminants, including ammonium perchlorate and polybrominated diphenyl ethers, on thyroid function. Their use in this capacity suggests that early life stage fathead minnows may be an adequate model for the screening of compounds with suspected thyroid hormone disrupting activity. However, no studies to date have examined the impacts of model thyroid disrupting compounds on this species. With this in mind, the objective of this study was to further develop the fathead minnow as a model for the screening of thyroid disruptors by identifying sensitive indicators of thyroid disruption in this species. Newly hatched fathead minnows were exposed to low, medium, and high doses of 6-propylthiouracil (PTU; a known thyroid inhibitor) or thyroxine (T4) for 35 days. On days 7, 21 and 35, fish were evaluated for size, general physical appearance and the expression of thyroid hormone related genes. Fish exposed to PTU experienced significant reductions in wet weight and length, decreases in pigmentation, alterations in body morphology and changes in the expression of four of ten thyroid related genes evaluated (trβ, ttr, di2, di3). In contrast, the only alterations noted in fish exposed to T4 were the expression of two thyroid related genes (ttr and di3). Taken together, these results suggest that developing fathead minnows may be well suited for assays aimed at detecting compounds suspected to inhibit thyroid hormone synthesis or signaling, but may be more limited in their ability to screen for thyroid hormone mimics.

571 Leveraging high-throughput screening data to define alternative adverse outcome pathways for impaired vitellogenesis

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Recent advances in high-throughput screening (HTS) have provided unprecedented information about the modes of actions of chemicals in the environment. Within the adverse outcome pathway (AOP) framework, this information is particularly useful for understanding molecular initiating events and early cellular responses that lead to apical effects of regulatory concern. For example, several endocrine modes of action (i.e., AR agonism, ER antagonism, aromatase inhibition) are linked to impaired synthesis of vitellogenin, the egg-yolk precursor, leading to reproductive impairment in fish and other oviparous animals. However, non-endocrine mediated pathways such as mitochondrial dysfunction, oxidative stress, and other degenerative changes in the liver can also impede the production of vitellogenin. These alternative mechanisms confound interpretations of the Fish Short-Term Reproduction Assay (FSTRA), which is used to identify endocrine-active substances in the USEPA Endocrine Disruptor Screening Program. The objective of this study was to leverage HTS data and identify compounds that act specifically through non-endocrine toxicity pathways for future AOP development. Twenty-eight assays related to mitochondrial dysfunction, oxidative stress, and endocrine disruption were compiled for 1797 compounds from ToxCast, transformed into nonparametric ranks, and imported into ToxPi. Compounds were further ranked by comparing ToxPi slice values for mitochondrial dysfunction and oxidative stress relative to endocrine signaling pathways. The analysis determined that many endocrine disrupting compounds, particularly androgen receptor agonists, also cause mitochondrial dysfunction and oxidative stress. Furthermore, nitrophenol, anthraquinone, and a subset of chlorophenol compounds were those most likely to impact mitochondrial function, while also having limited effects on endocrine signaling. Results from HTS assays were also compared for 9 compounds that decreased vitellogenin in the FSTRA, and it was determined that 4 compounds (Tetrachlorvinphos, Metolachlor, Flutolanil, Propargite) caused mitochondrial dysfunction or oxidative stress at lower doses than assays for endocrine disruption. These results suggest that mitochondrial dysfunction and oxidative stress are likely involved in the AOP for impaired vitellogenesis, and the chemicals identified through this novel computational approach should be investigated to define these alternative toxicity pathways.

572 Assessing the Potential Impact of an Amine Oxide Surfactant on Estrogenic, Androgenic and Aromatase Endpoints in a Fish Endocrine Screening Assay

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Amine oxides (AO) surfactants have a diverse set of applications with 95% of the volume produced in North America used in household cleaning products (e.g., dishwashing, laundry detergents and hard surface cleaners). Because of their wide-spread use they are classified as a high production volume class of compounds in several member countries by the Organizations for Economic Co-operation and Development (OECD). AO surfactants have a low potential to bioaccumulate in aquatic tissues and the AO that was tested demonstrated relatively low aquatic toxicity. These characteristics minimize the potential for environmental exposure and toxicity to aquatic organisms. The purpose of this investigation was to evaluate the potential for estrogenic, anti-estrogenic, androgenic and impacts to steroidogenesis from an AOsurfactant using a model aquatic vertebrate. Based on the non-cyclic structure of this AO surfactant, it was not predicted to be a ligand for the estrogen or androgen receptors or to disrupt steroidogenesis at environmentally relevant exposure concentrations and this is consistent with mammalian studies which have shown no reproductive and developmental effects. Fathead minnows were tested following the OECD 230 test guideline at concentrations of 0.14, 1.4 and 14 mg/L in a flow-through system after a 21-day continuous exposure. It was established in 14-day range-finding study that the highest test concentration did not result in overt toxicity. Endpoints used to assess activity were secondary sexual characteristics (tubercle scores) and vitellogenin levels (VTG). There were no effects on survival, growth, secondary sexual characteristics or VTG levels. Based on the endpoints evaluated, this AO surfactant has been shown to not have estrogenic, anti-estrogenic, androgenic effects or produce aromatase inhibition in fish, which confirms initial predictions based on its structures and results from mammalian studies for structurally similar AO surfactants. The no observed effect concentration (NOEC) was concluded to be 14 mg/L, a level that greatly exceeds predicted aquatic exposure concentrations.

573 Thinking outside the thyroid: Implications of disrupted thyroid hormone signaling on reproduction

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Traditionally, research into the effects of endocrine disrupting compounds (EDCs) has focused on one endocrine axis independent of all others. This approach may be shortsighted given evidence that alterations in signaling along one endocrine axis can affect signaling in others. For example, changes in thyroid hormone levels are known to alter reproductive function in a variety of vertebrates suggesting that thyroidal EDCs have the potential to alter reproductive function via novel mechanisms. Understanding thyroid-reproductive system interactions in model organisms, such as the fathead minnow (Pimephales promelas), is important when screening for EDCs and in establishing potential adverse outcome pathways for a compound. The objective of this study was to determine the reproductive outcomes of thyroid disruption as evaluated across multiple levels of biological organization (e.g., molecular, cellular and whole organism). Fathead minnows were exposed to the known thyroid disruptors polybrominated diphenyl ether 47 (PBDE-47), thyroxine (T4) or propylthiouracil (PTU) for 28 days via the diet. On day 7 of the exposure period, minnows were subjected to a 21 d breeding study during which time fecundity, clutch size, fertilization success and hatching success were evaluated. Upon termination of exposure, thyroid hormone- and sex steroid hormone-related gene expression, plasma sex steroid levels, gonadal histology, and sperm motility were evaluated. The results of this study revealed that thyroidal EDCs are capable of altering not only thyroid signaling in minnows, but also reproductive endpoints. For example, minnows exposed to PBDE-47 experienced significant alterations in the expression of thyroid-hormone related genes including deiodinase II and transthyretin confirming alterations along the HPT axis. In addition, females exposed to PBDE-47 were found to have significantly reduced gonadal expression of aromatase and estrogen receptor alpha than controls, while males were shown to have decreased plasma 11-ketotestosterone levels. The low binding affinity of PBDE-47 to sex steroid hormone receptors, combined with a lack of alteration in vitellogenin, suggest that the impacts of PBDE-47 on the HPG axis are the result of thyroid disruption, rather than direct interaction with sex steroid hormone receptors. Overall, this study shows that the impacts of thyroidal EDCs extend beyond the HPT axis to the HPG axis, which should be considered in EDC screening assays.

574 Reliance on whole animal tests to detect the reproductive effects of municipal wastewater effluents

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We conducted lab based experiments to determine the potential of effluents from different municipal wastewater plants to affect the reproduction of adult zebrafish. The endpoints measured included whole animal reproductive performance based on the numbers of eggs spawned over a 7-day exposure period and biomarkers of reproductive function including the levels of sex steroid hormones levels in ovarian tissue and the expression of selected genes in the ovary. In studies conducted in 2013 and 2015, a 50% dilution water collected immediately downstream of the wastewater treatment plant in Waterloo Ontario resulted in a significant reduction (50 and 85% in the two years) in the numbers of eggs spawned. In contrast effluent collected downstream of two different sewage treatment plants (Kitchener and Guelph, Ontario), an upstream reference site on the Grand River (Hespler) and the laboratory control water had no effects on spawning success. Measurement of ovarian testosterone and 17β-estradiol levels did not reveal an effect for fish exposed to the Waterloo effluent whereas zebrafish exposed to the Guelph effluent had lower levels of 17β-estradiol but only for the 2013 study. Expression of genes involved in steroid hormone biosynthesis including steroid acute regulatory protein, aromatase and the luteinizing hormone receptor also failed to reveal a consistent pattern of response. A reduction in aromatase expression was seen in fish exposed to the Waterloo effluent in 2013 but not 2015 whereas the other genes were not affected. Similarly fish from the Guelph site showed a reduction in aromatase in the 2013 but not the 2015 studies. We detected high concentrations of ammonia in the Waterloo effluent which we suspect is responsible for the reproductive impairment. Indeed other studies in which zebrafish were exposed to ammonia led to impairment in spawning success but inconsistent effects on steroid hormone levels or the expression of genes involved in steroid hormone biosynthesis. Collectively these studies suggest that the inclusion of endpoints of whole animal performance such as spawning success are a far more robust screen of reproductive toxicants than measurements of hormone levels or gene expression particularly when the underlying mechanisms of reproductive impairment are unknown.

575 Testing for Endocrine-Mediated Effects in Vertebrate Ecological Receptors: Potential for Read-across

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The U.S. Environmental Protection Agency (EPA) and the Organisation for Economic Cooperation and Development (OECD) have developed numerous tests to evaluate whether a chemical has the potential to interact with the endocrine system. Testing programs such as the EPA's Endocrine Disruptor Screening Program have purposefully included redundant tests to avoid false negatives. Given the impracticality of testing all man-made chemicals using traditional animal tests, predictive models and screening methodologies are needed. Approaches based on adverse outcome pathways (AOPs) may be advantageous in assessing species susceptibility and helping to prioritize chemicals for further testing. Certain axes, such as the hypothalamus-pituitary-gonadal (HPG) axis, are highly conserved among vertebrates; thus, there is a potential to use existing data for mammalian models to evaluate other vertebrates (fish, amphibians, birds, and reptiles). Read-across and computational approaches are promising for chemicals that interact with endocrine systems with known modes of action. We reviewed the HPG axis, hypothalamic-pituitary-thyroid (HPT) axis, hypothalamic-pituitary-adrenocortical axis, somatotropic axis, retinoid signaling pathway, vitamin D signaling pathway and peroxisome proliferator-activated receptor signaling pathway for homology and potential for read-across application. Important considerations for read-across and computational approaches include potency and internal dosimetry. Read-across and computational approaches that rely upon molecular or cellular assays may be difficult to interpret for chemicals that are activated through metabolism. In addition, conserved hormones may have different roles among vertebrates (e.g., prolactin controls osmoregulation in fish and lactation in mammals) and will need to be considered. The most promising read-across approaches for chemicals that interact with the endocrine system based on availability of data are between mammals and fish. Validation of read-across and computational approaches will be needed and the type and degree of necessary validation has yet to be determined. Future research should focus on filling data gaps to current read-across and computational approaches, instead of testing more species.

Passive Sampling in the Aquatic Environment: Recent Developments and Advances

576 Visibly-detectable Dyes as Performance Reference Compounds in Passive Sampling Devices

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Dozens of research efforts in the previous 10+ years have demonstrated that measurements made using Passive Sampling Devices (PSDs) represent precise and accurate estimates of available organic compounds in aquatic sediment, surface water, and soil. To overcome the challenges of impractical PSD deployment times in environmental matrices, the PSD research community has adopted the use of Performance Reference Compounds (PRCs) which are loaded into PSDs prior to deployment and used to infer the fraction of steady state attained during the deployment. Traditional PRCs have included compounds with a similar hydrophobicity to the compounds of interest such as stable isotope-labeled or deuterated forms of the compounds of interest or compounds that are not expected to be absorbed in significant amounts (e.g., rare Polychlorinated Biphenyl (PCB) congeners). Adding these PRCs to PSDs is expensive and requires complicated and time-consuming PRC measurement techniques (e.g., GC or HPLC methods). This presentation will highlight the application of a potentially more time- and cost-efficient suite of compounds for PRCs: visibly-detectable dye compounds. Compared to compounds traditionally used as PRCs, dyes are orders of magnitude less expensive, generally less toxic, can be added and extracted from PSDs in amounts easily measured via Ultraviolent/Visible (UV/VIS) spectroscopy or other visible/ colorimetric means, and can be observed in the PSDs with the naked eye. Measurement of dyes in PSDs can be performed with an inexpensive benchtop UV/VIS spectrophotometer via a non-destructive technique in a matter of seconds for a fraction of the cost to measure traditional PRCs. This presentation will detail the approaches and benefits of using dyes as PRCs in a commercially-available PSD, as well as empirical evidence that demonstrates the proof of concept. Our experiments in static and mixed PSD deployments in the laboratory, as well as deployments in the field, indicate the kinetics of dye PRC desorption match those of target analytes (and traditional PRCs) such that dye PRCs can serve as replacements for traditional PRCs. Overall, the use of dye PRCs will allow a much more streamlined and efficient approach for PSD research and development and will also enable more cost-effective analytical chemistry techniques.

577 Cross-validation of passive sampler measurements at the sediment-water interface by parallel use of PE and silicone sheets on a new porewater probe

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In situ data from passive samplers in water and sediment are often challenged by the fact that passive samplers do not reach equilibrium for all compounds within the deployment time. Another challenge is the availability and quality of polymer-water partition coefficients that are needed to convert measured concentrations in polymers to freely dissolved aqueous concentrations. We followed a new strategy of deploying two types of polymers in multiple thicknesses simultaneously to offer means of cross-validating passive sampler field data. To quantify chemical activity gradients over the sediment-water interface, we deployed µm-thin LDPE films and silicone PDMS membranes as passive samplers integrated in a recently developed sediment porewater probe that offers a 2.5cm vertical resolution of the contamination profile within the sediment. Based on the various thicknesses of the deployed passive samplers, the equilibration status of the samplers was assessed and compared to the results from dissipation rates of performance reference compounds (PRCs) that had been spiked into all polymers prior to field exposure. At the same time, it was possible to cross-validate estimated freely dissolved concentrations on the basis of both polymer-water and polymer-polymer partition coefficients for the two types of polymers. Finally, all in-situ sediment data were compared to ex-situ equilibrations with passive samplers of multiple thicknesses. Both silicone and LDPE seemed suited for sampling PAHs and alkyl-PAHs, PCBs and chlorinated pesticides. In conclusion, parallel deployment of thin sheets of LDPE and silicone in various thicknesses proved to be a simple and effective tool to validate obtained passive sampling data for a wide range of hydrophobic organic chemicals. At the same time, the results raise the question whether a PRC correction for non-equilibrium situations should be applied in all instances.

578 Method comparison for measurement of porewater Fe(II) and sulfide: in situ DET and DGT vs. ex situ centrifugation followed by colorimetric analysis

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In productive coastal sediments, the distance between the oxic and anoxic layers may be as little as a few millimeters. In such sediment the distributions of products of different respiration types can form a complex 'mosaics' resulting in iron(II) and sulphide being present at the same depth horizons on scales sampled by common methods. Many porewater sampling and analysis methods simply cannot distinguish changes over such small distances. Therefore, in sediment with complex, three-dimensional, time-dependent heterogeneity, it can be extremely difficult to examine the fine-scale (mm to sub-mm) chemical distributions of important chemical species using typical sampling and measurement methods. Typical porewater sampling techniques require the removal and processing of sediment cores. Porewater profiles are obtained by slicing the collected cores at 1 cm resolution, homogenizing the material and centrifugation of the section to extract pore waters, which are then analysed. This mixes the porewater solutes, which is likely to result in chemical reactions (e.g. formation of FeS) that change the solute concentrations considerably, making the final porewater concentrations measured inaccurate and non-representative. The diffusive equilibration in thin films (DET) and the diffusive gradients in thin films (DGT) techniques allow the in situ determination of elemental solute concentration profiles in sediment pore waters and also provide two-dimensional distributions at higher spatial resolution than is possible employing conventional techniques. This allows measurement of mm-scale features, facilitating the investigation of small scale biogeochemical processes and heterogeneity within the sediment. Furthermore, the in situ nature of the DGT and DET techniques limits inaccuracies caused by removal and processing of sediment pore waters. This study has obtained measurements of sediment porewater Fe(II) and sulphide co-distributions using conventional core sampling technique and new colorimetric passive sampler techniques. This study suggests that the colorimetric Fe(II)-DET and sulphide-DGT measurements are both more accurate and more representative than the conventional analysis of porewaters separated by centrifugation, even with the precautions taken against oxidation. This comparison will be an important validation step that will lead to the new techniques being accepted. Recent results of comparison of metal analysis will also be presented.

579 Strategies for transferring mixtures of organic contaminants from multimedia environments into bioassays

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Many of the chemicals that we use in our daily lives end up in multimedia environments including biota where they may pose a risk to ecosystems and human health as part of mixtures and together with their transformation products. Depending on the persistence and physicochemical properties of the organic contaminants, individual chemicals that make up the mixture partition and distribute within the environment and might then jointly elicit toxicological effects. For the assessment of the hazard potential and for the monitoring of such mixtures, a variety of cell-based in vitro and low-complexity in vivo bioassays based on algae, daphnids or fish embryos are available. Bioassays provide an integrative measure of the overall toxic potential of a sample and are hence complementary to chemical analysis that provides quantitative information on selected chemicals. However, the quantitative link between the exposure in the environment and that in the bioassay is not always taken into account. Establishing this link is difficult because different chemicals have distinct enrichment factors and sampling rates. Furthermore, they sorb to different extents to sorptive phases present in bioassays, which reduces exposure. The major obstacles to successfully applying bioassays for assessing environmentally relevant mixtures of organic pollutants are to collect, extract and dose the mixture into the bioassay without the mixture composition being altered while linking the observed effect back to the concentrations in the original medium. This presentation outlines various strategies of quantifiable transfer of organic contaminants from the aquatic environment including water, sediment and biota into bioassays covering sampling, extraction (exhaustive extraction vs. passive sampling) and dosing (solvent spiking vs. passive dosing) to transfer the mixtures into bioassays, while conserving or re-establishing their chemical composition at adjustable levels for concentration-effect assessment.

580 A Non-Selective Passive Sampling Device to Measure Both Polar and Non-Polar Organic Chemicals in Water

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The use of hydrophobic passive sampling devices (PSDs) such as low-density polyethylene (LDPE) and semi-permeable membrane devices (SPMDs) have proven reliable for measuring longer-term (e.g., 30-day) time-weighted average (TWA) exposure to hydrophobic chemicals with log KOW values above about 4.5. However, making similar TWA measurements for more polar chemicals with log KOW below about 4.5 have not been so reliable. The polar organic chemical integrative sampler (POCIS) was developed for more water soluble organic chemicals with log < 3.0, but the POCIS often does not provide reliable quantitative exposure estimates. There also remains a gap between what LDPE/SPMDs and POCIS can accumulate with chemicals in the log KOW range of about 3.0 to 4.5 not accumulating reproducibly in either device. We have developed a more universal non-selective passive sampling device (nsPSD), which is composed of a non-selective porous stainless steel cartridge or screen and a mixed-polymer sequestration sorbent. The nsPSD has been calibrated in the laboratory for over 500 organic chemicals with log Kow values ranging from 0.2-8.15. These chemicals include polychlorinated biphenyls, current-use pesticides, organochlorine pesticides, polycyclic aromatic compounds, pharmaceutical and personal care chemicals, algal toxins, and many metabolites and other degradation products of these chemicals. We have deployed these samplers at numerous field sites and in most cases the TWA exposure estimates using the nsPSDs closely match those using sequential grab sampling (every 1-2 days) over the time of deployment. We report here on the field verification of this new nsPSD using data from numerous Superfund Sites in the US. We believe this new nsPSD has the potential to significantly advance our capability to estimate chronic exposure of chemicals in water.

581 In situ equilibrium passive sampling of hydrophobic organic compounds in coastal marine sediments

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The study provides the first comprehensive dataset on in situ measurements of freely dissolved concentrations and chemical activities of PAHs and PCBs in coastal sediments of the North Sea and the Baltic Sea. During a research cruise in summer 2015, 50 in situ samplers were deployed in the coastal area of Germany. After three months 37 of the samplers were recovered. SPME fibers with different coating thickness (10 µm and 40 µm) were collected and analyzed in the laboratory. During both cruises surface sediment samples were simultaneously collected at the same sampling stations. For in situ sampling an equilibrium passive sampling device was used that facilitates the in situ measurement of hydrophobic organic chemicals (PCBs and PAHs) bioavailability in sediments in terms of freely dissolved concentrations. The sampler is applicable in a multitude of aquatic environments, especially where currents are low and sediments are muddy and well-mixed e.g. by bioturbation. Examples for such environments are mud flats, harbor basins, river banks and lakes. The field sampler allows PDMS fibers and hollow fibers to be immersed and equilibrated in situ, whereas an automated liner exchanger (ALEX) facilitates the quantitative transfer of analytes to the GC without the use of extraction solvents. Additionally, ex situ SPME experiments of these sediments were performed in the laboratory with the same analytical method. Experimental parameters like temperature and exposure time were adjusted as to meet the conditions of the corresponding experiment with the in situ sampling device. Finally, the results of the in situ sampling were compared with ex situ laboratory experiments. On the basis of the experimental findings, advantages and disadvantages of ex situ versus in situ measurements are discussed. From the in situ and ex situ experiments we examined (i) spatially resolved freely dissolved PAH concentrations (C_{free}); (ii) baseline toxicity potential on the basis of chemical activities (a); (iii) site specific mixture compositions and (iv) site specific distribution ratios.

582 Current Use Pesticides in Water Resources in Ontario, Canada: Can POCIS Passive Samplers Be the Basis for a Monitoring Program?

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Current use pesticides (CUPs) enter the aquatic environment from many point and non-point sources, including runoff from agricultural areas, turf farms, public spaces, golf courses and lawns and gardens, as well as discharges from wastewater treatment plants. Over the past five years, we have been developing a monitoring program to determine the distribution and sources of CUPs in watersheds impacted by both urban and rural sources of contamination. We have deployed Polar Organic Chemical Integrative Samplers (POCIS) in rivers and streams, the nearshore zone of Lake Ontario and in sources of raw drinking water. These monitoring efforts have shown that current use herbicides, fungicides and neonicotinoid insecticides are widespread in these aquatic resources. The herbicides, atrazine, diuron and 2,4-D, and the fungicides, carbendazim, thiophanate-methyl and a hydroxy-metabolite of chlorothalonil were widely detected at estimated time weighted average concentrations in the ng/L range. POCIS deployments in raw drinking water indicated that several neonicotinoid insecticides, including thiamethoxam, clothianidin and imidacloprid were present at low concentrations in the sources of drinking water for municipalities located in rural areas. Overall, these data indicate that there are point and non-point sources of CUPs that could affect aquatic life in receiving waters and contaminate sources of drinking water. However, it is currently unclear whether data generated from POCIS passive samplers are accepted scientifically due to uncertainties regarding appropriate methods for estimating sampling rates for these devices and approaches for applying Performance Reference Compounds (PRCs) to adjust sampling rates in the field. We will describe our experience with two methods for determining sampling rates for CUPs and for applying PRCs, and we will compare our results to data reported in the literature.

583 Answering the "So What?" question: Integrating toxicological databases into passive sampler studies

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The identification and quantification of chemicals in the environment has been the backbone of passive sampling studies for decades. Adding a biological effects component to those studies has required screening of extracts from passive samplers with a variety of in vitro and in vivo assays. Although informative, this requires testing one endpoint at a time which can quickly exhaust available sample volume and funding. In 2010 and 2014, semipermeable membrane devices (SPMDs) and polar organic chemical integrative samplers (POCIS) were deployed at a total of 69 sites in tributaries of the Great Lakes region. Extracts from these samplers were analyzed for a broad suite of legacy and emerging contaminants and these chemical data were compared to benchmark biological effect concentrations from the USEPA's ToxCast program. Numerous chemicals or chemical classes were frequently detected including atrazine, DEET, fragrances, phosphate-based flame retardants, PAHs, PCBs, chlordanes, endosulfan, and PBDE-47. The estimated time-weighted average water concentrations for many of these chemicals ranged from low nanogram per liter to low microgram per liter levels. Of the 170+ chemicals targeted in the SPMDs and POCIS, 66 were represented in the ToxCast database. Chemicals were screened for relative potential impact based on the ratio of concentrations in the passive samplers to the AC50 values (concentration at half maximal activity) from ToxCast. Although several chemicals exhibited some calculated activity, only a few of the PAHs were present at sufficient concentrations to suggest a potential for biological impact. This study demonstrated the utility of combining chemical data with toxicological databases to provide screening level toxicological information which can be used to direct additional in-depth study.

Complexity Kills the Minnow: Predicting the Ecological Consequences of Complex Pharmaceutical Mixtures

584 Unexpected drivers of low dose complex pharmaceutical pollutant mixture sublethal effects: when additivity in not enough

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Pharmaceutical pollution in freshwater systems has emerged as a major concern over the past decade. Currently, toxicity of these products is typically studied at high doses on a chemical-by-chemical basis under laboratory conditions. However, the identification of the true environmental impacts of pharmaceutical pollutants remains elusive due to complexity associated with their presence in "low doses" and "mixtures" in real world conditions. This presentation addresses the pervasive problem of low-dose pharmaceutical mixtures by developing and applying a novel experimental and statistical integrated analysis framework (GSA-QHTS). This framework combines Global Sensitivity Analysis (GSA) with Quantitative High-Throughput Screening (QHTS) as a cost-effective integrated framework that allows to screen main drivers of an experimental system respose when evaluated under a combined high-dimensional set of candidate drivers. This set of candidate drivers may include any chemical, physical or biotic factor/stressor that can be set up and control under laboratory experiments. We present a case study where GSA-QHTS allowed for the identification of the main pharmaceutical pollutants (and their interactions) driving non-linear/non-additive low-dose mixture effects at the microbial population level. Furthermore, the main pharmaceutical pollutants identified as important by GSA-QHTS at the population level were also found to be important at the community level in mesocosm experiments. Contrary to our expectations, the bioactivity of the mixtures was not predicted by the Concentration Addition additive mixture effect model. Our results suggest that we may be largely misidentifying most important emerging environmental pollutants under real world conditions.

585 Multi-Generational Exposure of Fathead Minnows to a Complex Urban Mixture of Contaminants of Emerging Concern

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Complex mixtures containing contaminants of emerging concerns are present in many Great Lakes tributaries. Using existing chemical and concentration data, the study assessed how an urban derived complex mixture affected multiple generations of fathead minnows (Pimephales promelas) exposed continuously under controlled laboratory conditions. Mature and larval minnows were exposed to a mixture comprised of Galaxolide (synthetic musk), TBEP (plasticizer), Estrone, Bisphenol-A (Plasticizer), DEET, Methyl-1H-benzotriazole (anti-icing agent), Desvenlafaxine (anti-depressant), Fexofenadine (allergy medication), Metformin (diabetes medication), and Nonylphenol at low (1/10th environmental), medium (environmental), and high (10x environmental) concentrations. F1 fathead minnows were exposed while sexually mature and produced F2 generation fathead minnows that were exposed throughout their entire life cycle. F3 generation fish were exposed during early development. Multiple endpoints were measured to assess the effects of the urban mixture on fish health and development. F1 minnows exposed to the urban mixture had higher plasma vitellogenin concentrations than control fish (mean: 2.73 ug/mL and 1.91 ug/mL respectively, $p< 0.05$). Exposure did not have a significant effect on male feeding performance, but it did reduce female feeding performance in the medium and high treatments (p-value: 0.01 and 0.03). F2 larvae had a greater predator avoidance escape angle when exposed to any mixture (p-values: 0.01, 0.02, 0.03). F2 larvae exposed to the high concentration were also smaller in size (p-value 0.01). The direct impact of reduced feeding efficiency and alteration in apical endpoints central to sustaining fish populations confirms that complex urban mixtures can adversely affect fish populations.

586 Molecular and behavioural responses of caged goldfish to pharmaceuticals and personal care products exposure in Cootes' Paradise

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Cootes Paradise is a wetland on the western tip of Lake Ontario, and is part of the Hamilton Harbour Area of Concern. Cootes Paradise is also an important site of biological diversity and a fish nursery. The primary source of contaminants in Cootes Paradise is the Dundas municipal sewage treatment plant (STP) which discharges in to the marsh. Pharmaceuticals and personal care products (PPCPs) have been associated with STPs and their recipient waters. Some PPCPs are known to cause endocrine disruption. To assess the endocrine disrupting potential of Dundas STP effluents to wild fish in Cootes paradise, male goldfish were caged for 21-days (5/cage, 10 cages/site) at three sites along a contamination gradient within Cootes Paradise. We also caged fish at a reference site in Lake Ontario: Jordan Harbour. Twenty male wild goldfish were captured from Cootes Paradise in the summer of 2012. Concentrations of PPCPs and profiles of endogenous proteins and metabolites were determined in plasma from both the caged and wild goldfish. Half of the caged fish were assessed in behavioural assays. This talk will (1) describe the observed behavioral changes and the protein and metabolite expression patterns of the caged fish, (2) relate those changes to the burden of PPCPs in the fish plasma, and (3) assess the ability of the caged fish to predict whether similar effects are likely in wild fish populations.

587 Potential metabolic disruption in juvenile Chinook salmon exposed to a mixture of CECs in the lab and field

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Our recent study assessed the occurrence and concentrations of a select number of chemicals of emerging concern (CECs) in water and 2 species of fish from 3 estuaries in Puget Sound, WA, USA including pharmaceuticals, personal care products (PPCPs) and industrial compounds. For samples collected over all sites, 81 analytes were detected in effluent, 25 in estuary water, and 42 in fish tissue, many at ng/g concentrations. Most of the detected compounds were antibiotics (15), neurological drugs (17), antihistamines, metabolic regulators, and several industrial compounds. Based on these data, we conducted a 50-day dose-response experiment with a dietary mixture of the most commonly detected analytes in fish tissue. The mixture included SSRIs, antibiotics, perfluorinated compounds, and drugs to treat diabetes, lipid imbalance, and hypertension. Fish were dosed for 32 days and fed clean food for 18 days. On days 32 and 50 we weighed fish and collected blood for assessment of 12 parameters including plasma glucose, triglycerides, lipase, cholesterol, and others as indicators of metabolic disruption. These blood plasma parameters were also quantified in juvenile Chinook sampled in the field from two local estuaries receiving wastewater effluent and one reference estuary. Significant differences were observed between impacted and unimpacted estuaries for several of the blood chemistry parameters. Additionally, significant differences for body mass and many blood chemistry parameters were observed in fish from the lab study that were exposed to the dietary mixture as compared to those in the control. The results from the laboratory and field studies will be presented, compared, and discussed. Supported by WA Department of Ecology G1300089 and NIEHS ES-04696.

588 Estrogenicity of wastewater and surface water: considering unknown compounds and mixture effects using an in vitro cell based assay

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Wastewater treatment plants are point sources of contamination and regulating the quality of these discharges using chemical analysis data presents many challenges, including the selection of relevant target contaminants and the lack of consideration of potential mixture effects. The use of in vitro biological tests (bioassays) is proposed as a complementary method to assess the potential risks associated with discharges of treated wastewater. The ERα-CALUX® (BDS, Amsterdam) was used as an in vitro bioassay to assess estrogenicity of environmental waters: influents and effluents collected three sewage treatment plants and impacted surface waters. The samples were prepared by solid phase extraction (Waters® cartridges MCX and MAX) and analyzed by liquid chromatography coupled to high resolution mass spectrometry (LC-HRMS) for the quantification of 18 indicator compounds, including known estrogenic compounds. The results indicated that effluents of the wastewater treatment plants had estrogenic activity (from 1.1 to 13.7 ng E2 EQ / L) significantly lower than their corresponding influent samples (from 26.6 to 173 ng E2 EQ / L). This estrogenic activity could not be accounted for based on the concentrations of estrogens detected: concentrations of 17α-ethinylestradiol (EE2) were usually below the detection limit. This suggests the presence of other compounds which contributed to the estrogenic activity. Estrogenic activities measured in surface water varied from 4.9 to 17.1 ng E2 EEQ/L, which are higher than the estrogenic activity trigger values of 0.1 to 0.4 ng E2 EEQ/L currently proposed in literature (Jarosova et al. 2014). These results underline the importance of using biological tests in addition to chemical analyzes in assessing the quality of treated wastewater and the potential impact of treated wastewater discharges on the receiving ecosystems.

589 Pharmaceuticals in Hudson River Water: Potential Toxicological Effects and Bioaccumulation in Aquatic Vegetation Using P. virginica as a Model

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Rapid increase of anthropogenic pollutants such as commonly used prescription drugs and pharmaceutical personal care products (PPCPs) is raising concern among the public and scientific community. Currently, there are no available methods to remove these compounds from wastewater, potentially affecting the environment and human health. In this study, the effects of fluoxetine, amphetamine, and triclosan on potential bioaccumulation, growth and development were investigated in controlled laboratory conditions using the aquatic plant arrow arum (Peltandra virginica) as a model. The following experimental treatments were established: 0.05 and 5.00 mg/L (fluoxetine), 0.002 and 0.2 mg/L ((S)-amphetamine), 0.0023 and 0.23 mg/L (triclosan). The concentrations were based on known ambient levels. Experimental treatments were dissolved in aquaria containing 23 L of raw (unfiltered) Hudson River water and three arrow arum specimens. There were two replicates of each treatment for 12 total experimental aquaria. Two controls were set up: a stationary control with raw Hudson River water, and a flow-through control having a continuous current of Hudson River water. After 18 days of exposure the experiment was terminated and both biomass and growth were recorded. Histological analysis was conducted on paraffin imbedded tissue sections. Total chlorophyll content was determined by cold extraction with N,N-Dimethylformamide. Spectro Genesis ICP-OES was used to analyze magnesium and calcium content. Presence of amphetamine was determined using a Torion Tridion 9 gas chromatography spectrometer. Histological analysis of leaf blades, petioles and roots showed signs of cellular deformation and lysis across treatments. Chemical analysis demonstrated an increase in chlorophyll content in triclosan treatments (80 mg/L) compared to control (30 mg/L). Additional chemical analysis suggested the potential bioaccumulation of (S)-amphetamine within the respective treatments. ICP-OES analysis showed decreased magnesium levels in low and high amphetamine exposure (483.3 and 581.9 µg/g dry weight respectively) in comparison to the flow-through control (736.4 µg/g dry weight). Gas chromatography revealed the presence of amphetamine in plant tissue. The results of histological and chemical analysis show pathological and physiological tissue alteration and suggest potential bioaccumulation of pharmaceuticals in aquatic vegetation.

590 Mixture Effects on Plant Uptake of Pharmaceuticals and Personal Care Products

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Municipal wastewater treatment plant effluents typically contain a variety of pharmaceutical and personal care product ingredients (PPCPs). In arid and semi-arid areas of the U.S. and other countries, reclaimed wastewater (RWW) is often used for irrigation, resulting in exposure of crop plants to complex mixtures of PPCPs. Crops irrigated with RWW may take up PPCPs through their roots, but the extent of their bioaccumulation in food crops is not well understood. Plants may respond to PPCPs in a concentration-dependent manner that affects their uptake, and some PPCPs may exert toxicity on plants. The effects of mixtures on plant interactions with PPCPs are not addressed in the current literature. Using the model plant Arabidopsis thaliana, we conducted root elongation assays to evaluate potential interactions between PPCPs that are commonly found in RWW. We identified several mixtures with effects of interest including synergistic toxicity when plants are exposed to both sulfamethoxazole and trimethoprim (antibiotics), and a reduction in toxicity when plants exposed to toxic levels of lamotrigine are also exposed to carbamazepine (antiepileptic drugs). Next, we conducted uptake experiments with single compounds and the identified mixtures at environmentally relevant levels. Uptake results from both A. thaliana and crop plants will be presented. Root elongation assays may be a useful tool for identifying compound combinations that affect PPCP bioaccumulation in crops plants.

591 In vitro estrogenic activity of representative endocrine disrupting chemical mixtures at environmentally relevant concentrations

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A wide range of natural and synthetic estrogen hormones, pharmaceuticals, pesticides and industrial chemicals may alter the function of endocrine systems and consequently cause adverse effects in wildlife and humans. These are commonly referred to as endocrine disrupting compounds (EDCs). Exposure to a mixture of EDCs has been hypothesized to have potential synergistic or antagonistic effects and can cause undesired effects that are not reflected by the individual compound. In this study, the estrogenic activities of 11 EDCs of global environmental concern were systematically investigated using the yeast estrogen screen (YES). The contribution of the individual chemical to the total endocrine activity of environmentally relevant mixtures was evaluated. For single chemicals, the relative estrogenic potency is, in order: ethinyl estradiol (EE2) > 17β-estradiol (E2) > estrone (E1) > estriol (E3) > 4-tert-octyl phenol (OP) > nonyl phenol (NP) > genistein (GEN) > bisphenol-A (BPA). Two chemicals, n-butyl benzyl phthalate (BBP) and dibutyl phthalate (DBP), showed only partial agonistic activity. Bis(2-ethylhexyl) phthalate (DEHP) showed almost no estrogenic activity in the YES assay. For binary mixtures using E2 as standard, E1, E3, EE2, BPA, GEN showed estrogenic effects, while DBP, BBP, DEHP, NP and OP showed anti-estrogenic effects. The full mixture of all these chemicals at an environmentally relevant ratio also showed weak estrogenic activity. The mixtures data were fitted to concentration addition (CA), response addition (RA) and interaction models (IR), respectively. The IR model is superior to the CA and RA models for the full mixtures containing both estrogens and antiestrogens. For the mixtures with the estrogenic group only, the mixture showed some additive effect, and results fit both CA and IR models well. Further, EE2 did not make a prominent contribution to the estrogenic activity of the mixture.

Differing Biotransformation Capacity Across Species: Measurements, Modeling and Implications for Decision Making

592 Toxicokinetics and biotransformation in aquatic invertebrates

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Pesticides, pharmaceuticals and other chemicals support our modern standard of life, but their use can have detrimental impacts on our environment. We studied the uptake, biotransformation and elimination (toxicokinetics) of pharmaceuticals in aquatic invertebrates because toxicokinetics determine the internal dose, which in turn determines toxicity. Here we present experimental evidence that biotransformation rates and pathways differ across different species of aquatic invertebrates. In particular we will present new data on ibuprofen toxicokinetics in Daphnia magna, Hyalella azteca and Lymnaea stagnalis. Further, we will place this new data in the context of recently published studies with other species and substances to reflect on the current understanding of biotransformation capacity across different species of aquatic invertebrates. Studying the comparative toxicokinetics of small, aquatic invertebrates is of great interest because it contributes to understanding evolutionary and environmental mechanisms and causes of differences in species sensitivity to chemicals.

593 Bioconcentration tests with fish and the freshwater amphipod Hyalella azteca. Are the results comparable?

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Bioconcentration factors (BCF) for regulatory purposes are usually determined by fish flow-through tests according to TGD OECD 305. Fish bioaccumulation studies are time consuming, expensive, and use many laboratory animals. Alternative methods that replace the use of fish for BCF testing would therefore be of value. The aim of this study was to investigate whether the freshwater amphipod Hyalella azteca can be used as alternative test organism for bioaccumulation studies, providing the opportunity to explain bioaccumulation from water (bioconcentration). Fourteen compounds of different lipophilicity (logKow 2.2 – 7.8) were tested under flow-through conditions to determine steady-state and kinetic bioconcentration factors (BCF_{ss} and BCFk). The results were compared with fish BCF estimates for the same compounds described in the literature. We could show that any of the compounds tested have no potential for bioaccumulation in H. azteca while being bioaccumulated in fish, i.e. no "false negative" prediction of bioaccumulation in fish. This means that the H. azteca bioaccumulation test could be an appropriate test to predict no B-vB classification (BCF < 2000) in the standard fish test. Bioaccumulation studies with H. azteca would support laboratory animal welfare considerations using a non-vertebrate species, improve efficiency and reduce costs for BCF-testing. However, a more detailed understanding of comparative metabolic profiles and metabolic activity when employing H. azteca, compared to fish, is needed. Preliminary studies carried out with H. azteca suggest CYP450 mediated bio-transformation comparable to fish. This is in accordance with the results obtained for other crustaceans e.g. Daphnia magna.

594 Variation among invertebrates in their capacity to biotransform sediment-associated hydrophobic organic contaminants

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Hydrophobic organic contaminants (HOCs) are generally assessed based on their potential to persist (P) in the environment, bioaccumulate (B) in biota and their potential toxicity (T). One challenge regarding hydrophobic organic contaminants HOCs is that they accumulate in sediments often to levels several orders of magnitude higher than in the overlying water, and that sediment assessment tools are scares as environmental risk assessment historically has focused on uptake from the water compartment. There are a number of papers illustrating that sediment-associated HOCs are available for uptake in benthic organisms, whereas there is considerably less information on the capacity of benthic invertebrates to metabolize organic contaminants (i.e., biotransform). These factors are likely to impact PBT assessment and categorization by, for example, adding uncertainty to predictions of accumulation and trophic transfer. Based on results from a number of experiments and literature values, this presentation will provide examples of 1) how biotransformation capacity varies among benthic invertebrates, 2) how biotransformation may reduce body burden and facilitate the removal of sediment-associated contaminants, and 3) compare aquatic vertebrate and invertebrate biotransformation capabilities.

595 Animal models in aquatic dietary toxicity testing: is there a suitable alternative?

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The need for non-animal based models is growing day by day due to regulatory requirements to reduce animal usage in toxicity testing. Until recently, in vitro intestinal models which can be used to address the dietary route of exposure were dominated by the mammalian models, with an absence of any comparable aquatic models. Recently, the development of the rainbow trout intestinal cell line (RTgutGC) has dramatically changed this by providing an aquatic alternative. This study presents data on the characterisation of this model in terms of metabolism and cellular change after exposure to two well characterised environmental pollutants, that of Benzo[a]pyrene and copper sulphate. These pollutants were carefully chosen due to previously reported uptake in the mid and posterior region of the fish intestine which is the region the cell line was developed from. Exposures were carried out in a saline solution after preliminary tests showed evidence of no significant damage to the cells using this carrier. Despite BaP being known to adsorb to various plastics/glass ware, active metabolism by the RTgutGC cells was verified both by fluorescent microscopy and GC-MS. Metabolic activity (EROD assay) between regions (mid and posterior) was assessed through a simple modification of solution pH, and indicated significant differences between regions based on pH when using the model toxicant BNF and BaP. BaP was shown to have a higher induction of CYP1A activity making it a more suitable CYP1A control for the intestine. Indeed further metabolic characterisation of BaP using the GST assay indicated while significant differences exists between pH's, BaP induced response in vitro plateaus above its known soluble range in vivo. This significant difference between regions is also evident in the copper uptake and is directly comparable to "gut sac" experiments presented in the literature for this fish and these regions. Not only does the cell line demonstrate active uptake via micro villi projections visible via TEM, but is also supported through staining for mucolytic substances. Analysis of gene expression at higher concentrations of copper also demonstrate comparable metabolic activity after metal exposure via increased expression in CYP3A, GST and Pgp. This study provides compelling evidence that the aquatic intestinal model derived from the cell line could potentially be used to supplement/replace dietary toxicity testing of specific compounds.

596 Physiologically based modeling of hepatic and gastrointestinal biotransformation in fish

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In fish, as in mammals, the liver generally viewed as the principal site of chemical biotransformation. For waterborne exposures, such as those conducted in support of standardized BCF testing, the effects of hepatic metabolism on chemical accumulation can be simulated using relatively simple models which assume, as a first approximation, that the liver operates on chemicals in blood, and that the blood is fully equilibrated with the rest of the animal. Such models may be inadequate, however, when biotransformation occurs elsewhere in the organism (e.g., gills or gut). This is particularly true when the diet is the primary route of exposure, as may be expected for hydrophobic chemicals in a natural setting, or when the organism is purposefully dosed in this manner (e.g., as part of recently developed dietary testing protocols). Here we use physiologically based toxicokinetic (PBTK) models to simulate how biotransformation occurrning in the liver and gut, alone or in combination, could be expected to impact chemical accumulation occurring as a result of water-only, diet-only, and combined water and dietary exposures. Of special interest was the desire to develop the simplest model capable of adequately representing these processes. To support this effort we developed a set of scaling factors needed to extrapolate in vitro activity in gut subcellular fractions to the intact tissue. The modeled results indicate the need to represent the liver and gut as tissues operating in series against a fraction of the total cardiac output. Failure to model these processes correctly could result in underestimation of either tissue's true impact on chemical accumulation, particularly when the model is used to solve for apparent rates of activity based on measured chemical residues in the organism or apparent dietary assimilation efficiencies. The contents of this abstract neither constitute nor necessarily reflect USEPA policy.

597 Are we there yet? How different fish species biotransform xenobiotics

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One of the greatest knowledge gaps in the study of xenobiotic metabolism is understanding how different species biotransform chemicals found in the environment. Fish are the most diverse group of vertebrates displaying a wide range of feeding modes and reproductive strategies and live in variety of environments. Biotransformation of xenobiotics also varies among fish species. Therefore, we compared xenobiotic metabolism of different fish species across a range of environmental conditions. We compared red and white-blooded Antarctic fishes (Notothenia rossii, Gobionotothen gibberifrons, Champsocephalus gunnari, and Chaenocephalus aceratus) that live in high dissolved oxygen(DO) concentrations and very low temperatures (T= 0^oC), alligator gar Atractosteus spatula that inhabits higher temperature waters (T > 30^oC) with low DO, and a wide range of salinities to rainbow trout Oncorhynchus mykiss, the reference fish that has been used for in vivo and in vitro metabolism and bioaccumulation studies. Antarctic fish were collected from the Elephant-South Shetland Islands region and the northern tip of the Antarctic Peninsula. Livers were frozen at -80 °C, transported to the lab, pulverized in liquid nitrogen and later homogenized on ice to prepare the liver S9 fraction for use in metabolism studies. Trout and garfish were maintained for 2 to 4 weeks under corresponding natural temperature and photoperiod regimes. Fish were euthanized and livers perfused with a buffered clearing solution (pH = 7.8), excised, homogenized, and centrifuged to obtain the liver S9 sub cellular fraction. The liver S9 fraction of the different fishes were used to perform metabolism studies with pharmaceuticals, B(a)P, and to determine conventional enzyme activities (EROD, GST). Our results indicate that the Antarctic fish species had significantly lower EROD and GST activities and no B(a)p metabolism when compared to trout. Alligator gar EROD and GST activities were similar to rainbow trout and exhibited significant metabolism of pharmaceuticals such as diclofenac. Alligator gar diverged from the teleost evolutionary line prior to the teleost genome duplication (TGD) and are therefore genetically more similar to tetrapods than are teleost fish, such as rainbow trout and Antarctic fishes. Alligator gar may possess unique adaptations to cope with xenobiotics.

598 Comparison of trout hepatocytes and liver S9 fractions as in vitro models for predicting hepatic clearance in fish

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Isolated hepatocytes and liver S9 fractions have been used to collect in vitro biotransformation data for fish as a means of improving modeled estimates of chemical bioaccumulation. To date, however, there have been few direct comparisons of these two methods. In the present study, cryopreserved trout hepatocytes were used to measure in vitro intrinsic clearance rates for 6 polycyclic aromatic hydrocarbons (PAHs). These rates were extrapolated to estimates of in vivo intrinsic clearance and used as inputs to a well-stirred liver model to predict hepatic clearance. Predicted rates of hepatic clearance were then evaluated by comparison to measured rates determined previously using isolated perfused livers (IPL) from well-matched animals. Hepatic clearance rates predicted using hepatocytes corresponded closely to measured values (< 2.1 fold difference for 5 of 6 compounds) under two competing binding assumptions. These findings, which may be attributed in part to high rates of PAH metabolism, are similar to those obtained previously using data from liver S9 fractions obtained from animals closely matching those used for the hepatocyte assays and IPL studies. For one compound (benzo[a]pyrene), the in vivo intrinsic clearance rate calculated using S9 data was 10-fold higher than that determined using hepatocytes, possibly due to a diffusion limitation on cellular uptake. Generally, however, there was good agreement between calculated in vivo intrinsic clearance rates obtained using either in vitro test system. These results suggest that both systems can be used to improve bioaccumulation assessments for fish, particularly when vitro rates of activity are relatively high, although additional work is needed to determine if the chemical domain of applicability for each system differs. The contents of this abstract neither constitute nor necessarily reflect USEPA policy.

599 Comparisons of in vitro, in vivo and in silico biotransformation rates in fish and humans

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Chemicals are being evaluated for Persistence, Bioaccumulation and Toxicity (PBT) properties and high-throughput models are being developed and applied for hazard-, exposure- and risk-based screening for prioritization. Biotransformation is a crucial process that can influence total elimination half-life and hence the relationship between chemical intake rate and internal concentration. Biotransformation is a key mitigating process for hydrophobic chemicals that could otherwise exhibit high bioaccumulation potential in aquatic and terrestrial organisms. Biotransformation can also be relevant in the context of toxicity due to bioactivation (e.g., PAHs). In the absence of measured in vivo data, in vitro and in silico models are often required to address data gaps. Despite the fundamental value of biotransformation rate information, relatively few measured in vivo data are available compared to the thousands of chemicals requiring evaluation. Our objective is to compile, evaluate and compare existing in vivo, in vitro and in silico data streams for estimating biotransformation rates for organic chemicals in fish and mammalian species. We have compiled a large database of in vitro assay estimates of metabolism (half-lives, clearance rates and rate constants) derived from liver microsomes, S9 fraction, and hepatocytes from on-line databases and the scientific literature. The in vitro database consists of 19,634 entries for 11 species (56% human data). We have also developed databases for whole body (in vivo) biotransformation rate constant estimates for approximately 940 chemicals in humans and 700 organic chemicals in fish. In vitro to in vivo extrapolation models are being developed and applied to the in vitro data to obtain estimates of hepatic clearance, and as applicable, whole body biotransformation rate (clearance, or half-life) estimates. In vitro biotransformation rate estimates and in silico predictions from existing screening-level quantitative structure-activity relationships (QSARs) are compared to in vivo biotransformation rate estimates. Key uncertainties and challenges comparing the datasets are described and a strategy to address data gaps and uncertainty is discussed.

Neonicotinoid Insecticides: Current Research on Fate and Effects

600 Water Quality Assessment of Potential Imidacloprid Impacts in Great Smoky Mountains National Park

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Imidacloprid treatments are used for the suppression of hemlock woolly adelgid (Adelges tsugae [Annand]) (HWA) (Hemiptera: Adelgidae) in Great Smoky Mountains National Park (GRSM). The presence of imidacloprid and possible effects on aquatic macroinvertebrate communities in streams associated with HWA imidacloprid treatment areas were assessed as part of a retrospective analysis GRSM's HWA management program, Ten conservation areas in which hemlocks in riparian areas were given soil imidacloprid applications were selected. Stream sampling sites were chosen upstream and downstream from each conservation area, for a total of ten paired upstream – downstream sites. Baseline aquatic macroinvertebrate data collected before imidacloprid use in GRSM were available. Surface water samples and aquatic macroinvertebrate samples were collected from each sampling location. Concentration of imidacloprid in parts per trillion (ppt) was determined using liquid chromatography mass spectroscopy (LC/MS). Aquatic macroinvertebrate communities were assessed as indicators of water quality. Imidacloprid was detected in seven of ten downstream locations. Concentrations ranged from 28.5 to 379 ppt, which are below USEPA chronic and acute aquatic macroinvertebrate aquatic life benchmarks. Aquatic macroinvertebrate taxa was similar at downstream sites compared to both upstream sites and baseline data. While potential impacts of HWA imidacloprid treatments on surface water quality are minimal, the extent of treatments should be carefully considered for overall system health.

601 Residue of the neonicotinoid imidacloprid in greenhous and landscape plants and effects on beneficial insects

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Integrated Pest Management (IPM) is a decision making process used to manage pests that relies on many tactics, including cultural and biological control, which are practices that conserve beneficial insects and mites. Neonicotinoid insecticide use was embraced by IPM as it was applied to the soil and not sprayed on foliage and was thought to reduce drift and nontarget effects on foraging beneficial insects on flowers and leaves. However, systemic, soil-applied neonicotinoid insecticides are translocated to pollen and nectar of flowers, often for months, and may reduce survival of flower-feeding beneficial insects. Neonicotinoid insecticide residues in pollen and nectar differ widely depending on the amount that is applied to crops and landscape plants. Seed treatments result in relatively low levels, less than 10 ppb in pollen and nectar from an application of 1.2 mg AI imidacloprid on corn seed or 0.05 mg AI on canola seed. The imidacloprid field crop rate is 4 mg/ sq. ft. A soil application of imidacloprid on a greenhouse label is 300 mg/12L pot. This is a 250 times higher rate when compared to a seed treatment rate on corn and a 75 times higher rate when compared to a field crop rate. Rates for trees are higher with 14 g deposited on the soil surface and 2.8 g injected into 10 in DBH trees. Imidacloprid residue in flowers for 5 species of herbaceous plants, rose, and linden trees from label rate applications are high. Translocation of imidacloprid from soil (300 mg AI) to flowers of Asclepias curassavica and Agastache foeniculum resulted in residue of 1500 to 6,030 ppb. Residue from pollen cut from treated flowers resulted in residue of around 100 ppb which is the amount that would alter behavior and colony health of bumblebees. Tree injections resulted in 1300 ppb and soil drenches 81 ppb in linden flowers. Greenhouse label rates of soil applied imidacloprid altered behavior and cause mortality in 6 species of beneficial insects and bumblebees, Bombus impatiens. In bumblebees, foraging and food consumption was reduced and resulted in less stored nectar and lower queen survivorship.

602 Effects of neonicotinoid seed-treatment use on non-target native pollinator communities in Missouri field margins and agricultural fields

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Neonicotinoid insecticides are the most widely used insecticide class in the world. Pervasive use of neonicotinoid seed treatments (e.g., corn, soybeans) across vast agricultural regions has led to increased scrutiny due to potential toxicity and sub-lethal effects on non-target organisms such as beneficial insects (e.g., bees). Neonicotinoids are persistent, highly water soluble, and have been shown to be transported via surface run-off and wind deposition of dust. Previous terrestrial research has focused on neonicotinoid effects on introduced species such as honeybees and to a lesser extent wild species such as bumblebees. However, it is unclear if neonicotinoid use reduces native pollinator abundance, diversity and/or alters ecosystem services (e.g., pollination, seed production) within agroecosystems. As numerous wild bee populations nest in the ground and forage on a range of flowering plants, it is unknown if many native bee species are equally exposed to neonicotinoids through transport and accumulation in soils and plants surrounding cropped fields (i.e., field margins). To evaluate effects of neonicotinoid exposure on native pollinator communities, we sampled 30 agricultural fields planted with treated or untreated seeds on five conservation areas throughout Missouri from pre-seeding through harvest. We hypothesized that native pollinators would be more abundant and diverse in fields never treated with a neonicotinoid compared to annually treated fields. We collected soils from agricultural fields and their associated field margins, sampled herbaceous and woody flowering species in field margins, and surveyed and collected a wide variety of native pollinators including bees and butterflies over time in fields and field margins. Here, we present our preliminary findings and discuss how this research improves our understanding of the potential impacts of neonicotinoid seed-treatment use on non-target native pollinator communities in agroecosystems.

603 Population Trends and Neonicotinoid Pesticide Exposures in Hummingbirds

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Fourteen species of hummingbirds co-occur in North America, and among the seven with reliable data, three species are showing significant population declines in populations as indicated by analysis of the North American Breeding Bird Survey BBS. The steepest negative trends annually are for pacific coastal Rufous Hummingbirds and Allen's Hummingbirds although Broad-tailed Hummingbirds are also declining. There may be multiple factors underlying those declines and stressors may act at different life stages and locales throughout the year as hummingbirds migrate, breed and overwinter. We examined neonicotinoid pesticide exposure in hummingbirds using agricultural regions in the Fraser Valley, southern British Columbia, and within the core of Rufous Hummingbird range. We quantified hummingbird presence and use of blueberry fields that were conventionally sprayed or organic and compared them to natural areas. While hummingbirds were commonly present in hedgerows of farms, nectaring on blueberry blossoms was not commonly observed. However, neonicotinoid insecticide concentrations were detected in blueberry flowers a year post spray and detected at 3 ng/g in pooled samples of cloacal fluid of Rufous and Anna's hummingbirds collected within 0.5 to 1 km of conventionally sprayed blueberry fields.

604 Consistent Patterns of Toxicity of the Nitroguanidine Neonicotinoid Insecticides to Honey Bee (Apis mellifera) Colonies

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In response to ongoing registration review activities in the US and Canada, studies were conducted on free-ranging honey bee (Apis mellifera) colonies fed sucrose solutions treated with either imidacloprid, thiamethoxam, or clothianidin. Each study consisted of five test concentrations in which treated sucrose solution was provided twice a week for six weeks during the summer. Each study was replicated across 12 separate apiaries in an area with low agricultural activity (central NC, USA). Colonies were assessed before, during, and after the treatment period and again the following spring. Across all the tested compounds, pollen stores and capped brood were the first and most affected endpoints with reductions beginning after three weeks of treatment. In subsequent assessments, reductions in adult bee strength, eggs, and open brood occurred and were likely downstream of initiating effects. These effects were characterized by concentration-response curves with a tendency towards recovery after the treatment period ended. Assessments at later time points did not result in effects at lower test levels indicating that the most significant effects occur during or proximal to the exposure period. Likewise, impacts on overwintering were not observed to be more sensitive. The results of these studies, carried out with a harmonized testing protocol, generated hazard values integral ot higher tier honey bee risk assessment. In addition, these studies allow for direct comparisons across the nitroguanidine neonicotinoid insecticides revealing consistent patterns of chronic effects which may be driven by reductions in pollen stores.

605 Assessing variation in neonicotinoid toxicity to freshwater invertebrates using species sensitivity distributions

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Neonicotinoids, a group of water-soluble pesticides commonly used in agriculture, have the potential to be toxicants to aquatic species. Sensitivity to neonicotinoid toxicity varies greatly among aquatic invertebrate taxa. Here we present the results of acute and chronic toxicity tests for six neonicotinoids: acetamiprid, clothianidin, dinotefuran, imidacloprid, thiacloprid, and thiamethoxam, for several aquatic invertebrate taxa including Ephemeroptera (Hexagenia sp., Caenis sp., Siphlonurus sp., Ephemera guttulata, Neocloeon triangulifer, Stenonema sp., Isonychia sp.), Hemiptera (Corixidae sp.), Diptera (Aedes sp., Chironomus dilutus), Odonata or damselflies (Coenagrion sp.), Amphipoda (Hyalella azteca), and Cladocera (Daphnia magna, Ceriodaphnia dubia). Standard model organisms were cultured under laboratory conditions and others were field-collected. Lentic invertebrates were exposed under static conditions, while lotic invertebrates were exposed using a recirculating apparatus that simulated a stream flow. All acute exposures were 48 to 96 hours in duration. Using these data, in conjunction with literature data, we plotted species sensitivity distributions (SSDs) to model the variation in neonicotinoid toxicity. 96-hour LC50 values ranged from 2 – >100 000 µg/L, with cladocerans being the least sensitive. Chronic (>21 day) growth and reproduction tests were conducted with select neonicotinoids for sensitive species (H. azteca, C. dilutus, N. triangulifer) and relatively insensitive cladoceran species (D. magna, C. dubia). Together with literature data, we used this chronic dataset to generate a long-term SSD for select neonicotinoids. This study is in partnership with the Ontario Ministry of the Environment and Climate Change, and will help to develop a comprehensive dataset from which to derive water quality criteria for the protection of aquatic life.

606 Detecting the sublethal effects of imidacloprid for bumble bee (Bombus impatiens) workers

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The agriculture industry relies on pesticides for crop production, but growing evidence suggests that sublethal effects of pesticides, in addition to other stressors, may contribute to the decline of insect pollinators. Neonicotinoids are a widely-used, advanced class of insecticides that are incredibly effective for crop protection but, at low levels, can have pronounced sublethal effects on bees. Our understanding of bee toxicology comes primarily from studies on honeybees, and the few studies on bumblebees (genus Bombus) have assessed toxicity on a colony level. We investigated how imidacloprid, a first generation neonicotinoid, affects the diet consumption, long term survival, and activity of individual bumblebees (Bombus impatiens) under different exposure scenarios and at a range of concentrations. We saw no significant effects when continuously fed 10 ppb imidacloprid in nectar but at 32 ppb, we saw a significant effect on diet consumption, long term survival (χ^2=6.042, p< 0.05), and activity (χ^2=13.89, p< 0.001). Knowing that 32 ppb produced effects with all three metrics, we looked at whether access to clean nectar following each day of exposure to 20 ppb imidacloprid would diminish the effects seen with continuous exposure. We saw a slight recovery in diet consumption and activity after the first day of clean nectar, however, after a second day on 20 ppb imidacloprid, we no longer saw this recovery. When individuals were exposed under different temperature conditions, those exposed at 16°C and 30°C showed elevated levels of activity and increased rates of diet consumption. Effects were seen within one hour of exposure with sustained elevated activity at 30°C and a gradual onset of immobilization at 16°C following a brief window of hyperactivity. Our data suggests that 1) individuals exposed to imidacloprid concentrations exceeding roughly 20 ppb may be behaviorally impaired, 2) these effects are dependent upon the temperature in which individuals are exposed, and 3) provided with clean nectar, individuals exposed to these concentrations are able to recover.

607 Effect of sublethal exposures of adult Northern Leopard Frog and larval African Clawed Frog to clothianidin and thiamethoxam

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Detections of neonicotinoid insecticides (NEO) in global surface waters often exceed water quality guidelines for aquatic species. Amphibians, which breed and develop in wetlands and ephemeral ponds, may be considered sentinel species in aquatic habitats because crucial phases of their development occur in water, and they generally do not venture far from where they were hatched. As such, they are valuable indicators of environmental stressors. To explore potential sublethal effects of two NEOs, clothianidin (CLO) and thiamethoxam (THX), African clawed frog, Xenopus laevis, tadpoles (n= 20/treatment) were exposed for 21 days to Belay® containing active ingredient (a.i.) CLO at high (100 ppm) and low (20 ppm) concentrations and to Platinum 75 SG® with a.i. THX at the same concentrations. Adult Northern Leopard Frog, Lithobates pipiens, (n= 20/treatment) were exposed to CLO and THX at high (100ppm) and low 20 (ppm) concentrations by virtue of an implanted carrier compound that provided for slow release delivery imitating a long-term chronic exposure of the a.i. over 28 days, with both carrier and uninjected control treatments. The mode of action of the a.i. is interference of neurotransmission at the conserved nicotinic acetylcholine receptor (nAChR), thus the hypothesis was that sublethal effects of NEOs could occur across taxa and at different life stages of amphibians. A biomarker approach allowed for quantifying responses. Hepatosomatic indices were obtained and genomic biomarker endpoints for tadpoles included RNA expression measured by quantitative real-time polymerase chain reaction of cytochrome P450 1A from livers and alpha-7 subunit of nAChR from brains. Genomic biomarker endpoints for the adults were the same, and included nAChR expression from testes. Cellular biomarker endpoints for adults included whole blood DNA fragmentation (an indicator of genoxity) and liver enzyme activity, both measured by flow cytometry. Adult brain acetylcholine was measured (an indicator of neurotransmission), and hepatosomatic- and gonadosomatic indices were generated. Sperm quality parameters and antibody-stained nAChR on sperm of adult frogs in this study and from other studies on honeybees implies NEOs may influence reproductive processes. Indications of NEO-induced stress were evident in both the tadpole and adult amphibians.

Alternative Assessments Best Practices for Safer Chemistry

608 Advancing the Applicability of Alternatives Assessment for Engineered Nanomaterials

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The future design of products incorporating nanomaterials and the redesign of existing products using engineered nanomaterials with an emphasis on reducing the potential hazards for human health and the environment is key to the success of the innovations made possible through nanotechnology. Alternatives assessment has the potential to be used as a tool for product designers to ensure the safety of products being developed. However, there is a need to assess the overall applicability of alternatives assessment methods for nanomaterials as these methods were originally developed for the evaluation of chemicals, not materials. This presentation reviews an assessment of the overall applicability of alternatives assessment for nanomaterials, focusing on the hazard assessment and exposure characterization components. The review focuses on several issues such as whether the novelty of these substances precludes the utility of an alternatives assessment approach, complexities involving the lack of an analogous Chemical Abstract Service (CAS) system for nanomaterials, differences in hazard assessment metrics for bulk chemicals versus nanomaterials, and differences in the environmental fate and transport of nanomaterials compared to chemicals that complicate existing exposure models. Several recommendations and suggested modifications to existing alternatives assessment frameworks that aid in the selection of safer nanomaterials were identified and will be reviewed, including: the critical importance of a functional use focus, safer nanomaterial design principles that reveal inherent hazard assessment criteria, and the use of control-banding techniques for exposure evaluation.

609 Use of Automated Tools to Facilitate Rapid Chemical Hazard Assessment of Consumer Product Chemicals

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There is a growing need on the part of consumer product manufacturers and their suppliers to rapidly assess large numbers of chemicals for human and environmental hazard. This need is increasingly apparent in the area of chemicals used in articles such as plastics, textiles, coatings, adhesives, and other common consumer product materials for a variety of applications. Many chemicals have not undergone toxicological testing and therefore often lack experimental data to help to determine characteristics of human or environmental concern or preference. Computer software can enable clustering of chemicals by similar molecular structures, then evaluate the chemicals within each group for representative toxicity data, and apply a read-across process to fill data-gaps within the group. Our project examined the utility of chemical structure clustering software combined with aggregated available chemical hazard data for a subset of the chemicals of interest in facilitating rapid assessment of chemicals with limited human and environmental health data. Our team used the EPA Chemical Assessment Clustering Engine (ChemACE) tool to cluster chemicals into groups with similar structural characteristics. Our dataset consisted of approximately 2200 chemicals and chemical-specific SMILES notation to indicate molecular structure. 1100 of these chemicals fit into 223-257 clusters, depending on the ChemACE settings used. Our team then evaluated clusters for hazard characteristics using the SciVeraLENS® automated chemical assessment platform. This combination of platforms allowed us to compare the chemicals with experimental hazard data and determine if they could be used to model the hazards of chemicals within the same cluster that do not currently have experimental data available. Our conclusions were: 1) In clusters where a chemical was high hazard for a critical endpoint (e.g., carcinogenicity, mutagenicity, developmental toxicity), the process highlighted the need for careful evaluation of the other chemicals in that cluster; 2) The results clearly indicated hazard characteristic trends across a cluster allowing for higher confidence in the use of analogous data, and 3) Having the ability to view the chemical structures in ChemACE for the compounds in the cluster was helpful for understanding subtle differences that could impact potential toxicity.

610 Data-Driven Assessments for Data-Poor Chemicals: Expanding the USEPA's Safer Chemical Ingredients List

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Since its inception in 2012 with 500 chemicals, the Safer Chemical Ingredients List (SCIL) has served as a resource for consumers and other purchasers by disclosing the ingredients formulated in Safer Choice-labeled products. Currently the list contains over 750 chemicals and is regularly expanded to reflect new chemistries used in labeled products and other substances that meet the rigorous Criteria for Safer Chemical Ingredients. Not only do these activities support the expansion of the Safer Choice program, but also help to expand the use of safer chemicals across the market. New candidates for the list were identified during a re-evaluation process of currently listed chemicals. Additional candidates were identified by evaluating other chemicals used in consumer products that are analogous to those chemicals currently listed on the SCIL. A candidate for the SCIL must meet criteria for multiple human health and ecological endpoints that prioritize the use of measured data. But data gaps for these substances are prevalent, complicating these data-driven assessments. To reduce uncertainties when assessing candidate chemicals, a cluster or category approach is often implemented. This approach incorporates several structurally-similar chemicals to aid in the filling of data gaps using read across and is often used for human health endpoints and ecotoxicity endpoints where reliable Quantitative Structure Activity Relationship (QSAR) estimates cannot be obtained in a transparent manner. The cluster approach has the added benefit of increasing certainty in the assessment by maximizing the integration of experimental data. This presentation will discuss the incorporation of cluster and QSAR approaches into assessments for new chemical additions to the SCIL, as well as the re-review process for existing SCIL chemicals. Examples illustrating the development and assessment of SCIL re-reviews and candidates will be included, as well as a summary of the "state of the data" available for these assessments in order to highlight the current challenges in identifying safer chemistry alternatives. The views expressed in this abstract are those of the authors and do not represent U.S. Environmental Protection Agency policy or endorsement.

611 Data Mining To Answer Complex Environmental Questions

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Like most fields of endeavor, Environmental Science has begun to produce volumes of data larger than can be understood or managed by traditional techniques. At the same time, Environmental Science seeks to understand complex systems at multiple temporal and spatial scales. Data mining of these new, large data sets is an ideal approach to answer complex questions. Our research group has pioneering the use of large data sets to answer questions about where chemical contaminants come from, how they are processed in watersheds, how and where they are degraded, and how long they might persist. This presentation will use case studies to describe the kinds of data that are publicly available and what questions these data can answer. We will describe some of the major methods we have used to parse large data sets to investigate patterns of organic pollutants in air, water, and sediment. Chemical classes we have investigated include Polychlorinated Biphenyls (PCBs), Polychlorinated Dibenzo-p-dioxins and –furans (PCDD/Fs), Polybrominated Diphenyl Ethers (PBDEs), chlorinated ethylenes, chlorinated benzenes, and antibiotics. This approach has allowed source apportionment across different manufactured formulations (such as Aroclors) and has identified processes (such as microbial dehalogenation and volatilization) and sometimes even surprising, inadvertent sources (such as PCBs from pigments). This presentation will describe some of the main challenges of applying data mining techniques to a variety of data sets from the east coast (Delaware River and New York/New Jersey Harbor) to the west (Portland, OR; San Francisco Bay), and will suggest future directions in data mining to answer complex environmental questions.

612 Building Your Toolbox: Read-across Tools for Alternatives Assessment

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Alternatives assessment (AA) provides a framework for evaluating chemicals of concern and their possible substitutes. The goal is to reduce inherent chemical hazard in order to address unforeseen exposure scenarios. However, incomplete health effects data may limit the ability to evaluate safer alternatives. Read-across approaches are increasingly used to address such data gaps. The available computational tools and approaches to support read-across vary in terms of sophistication, ease of use, and comprehensiveness, leading to analyses of variable quality and reliability. There are two critical steps in a robust read-across methodology: 1) quantifying structural similarity between proposed surrogates and a target chemical, and 2) showing an expected common mode of action (e.g. via structural alerts). To explore the utility of different analytical tools, we established a set of 37 chemicals with structural similarity to a target chemical – the established skin sensitizer hydroxyethyl acrylate. We first evaluated chemical similarity between individual chemicals and the target using two computational tools, ToxMatch and ChemMine. After ranking the chemical test set by similarity score, the two programs demonstrated statistically significant agreement (Kendall's coefficient of concordance, $W = 0.85$, $p = 0.005$). We also evaluated the dataset for skin sensitization structural alerts using the structure-activity relationship (SAR) programs Toxtree and Derek™, and compared the predictions to animal test data obtained using standard assays. These programs in combination exhibited a sensitivity of 85% (correct prediction of hazard for 17 out of 20 animal test positives), and a specificity of 89% (at least one program did not report an alert for 16 of 18 animal test negatives). For read-across, we refined our surrogate selection approach by considering physicochemical data relevant for skin sensitization (e.g., molecular weight, water solubility, and vapor pressure). This improved the overall accuracy of our read-across approach by 49% (51% vs. 100% accurate prediction rate). These results are consistent with those from an earlier evaluation of 67 possible chemical analogues for p-phenylenediamine, another skin sensitizer, suggesting that read-across can be used to bolster AAs when multiple tools and information sources are carefully implemented to fill data gaps and reduce uncertainty.

613 Improved Read Across in Alternatives Assessments

H. Plugge, 3E Company

Data quality and quantity has always been the limiting factor in Alternatives Assessment. Given the newly legislated need for screening techniques, input data becomes ever more important. Data curation for screening has major limitations: the increase in data quality is often not worth the investment for screening purposes. Various simple statistical techniques can be used to improve the overall uncertainty in uncurated data – examples will be provided of uncurated vs curated data and best practice estimates of screening input data. Using (semi) curated data will allow a faster screening of read across of datapoints for alternatives assessment screening. We will discuss overall hazard screening of an analog series of aldehydes and ketones, using 3E Green Score methods. Using these data one can identify datagaps and anomalies. Both of these can be addressed (to some extent) by performing a read across matrix. This matrix approaches uses both analogues and homologues to provide continuous series of matrices for various endpoints. Often only selected data is missing within these matrices. Both overall screening assessments and drill down data will be provided allowing the assessor to visually appraise the need for filling in datagaps and addressing the most useful matrix comparison per endpoint. Examples will be provided of the actual filling in of datagaps using these matrices. Notably given their overall reactivity, aldehydes are often less toxic that the corresponding ketones. Some of this is perhaps due to different metabolic pathways, e.g. hexanal vs 2-hexanone. Different isomers will also react very differently , an example being 2- vs 3-hexanone which have very different postulated metabolic pathways, although it is unclear which pathways are in use at the often high test doses.

614 Real-World Implementation of Alternatives Assessment Resulting in Safer Chemical Substitutions

C.D. Robertson, Hewlett-Packard

A business case for screening alternatives using alternatives assessment based on the cost savings realized by avoiding multiple substitutions will be discussed. Selection of inherently less hazardous alternatives as the first step in the assessment process reduces the risk that the substitutes will face future restrictions. Examples of the inherent benefits of this green chemistry approach will be presented. Transitions away from chemicals of concern to safer alternatives is difficult and expensive, especially in a complex supply chain. Proactive use of alternatives assessment on emerging chemicals of concern can help manufacturers prepare to replace these chemicals with better alternatives. The trigger for actually making the transition has traditionally been regulation but other motivating factors are emerging. Recently, alternative assessment and hazard assessment have been incorporated into ecolabels and certification criteria. The U.S. Green Building Council's LEED certification now incorporates hazard assessment into building material requirements. An important ecolabel for the electronics industry is TCO Development's TCO Certified label. TCO has incorporated an accepted substance list for flame retardants based on the GreenScreen™ for Safer Chemicals. In the United States, EPEAT certification is a "Green Public Procurement" requirement that must be met before bidding on many government purchasing contracts. Alternatives assessment is being discussed for inclusion in the next revision of the EPEAT standard and should be complete by the time of this presentation. Aspects of these ecolabels will be presented as well as a discussion of their ability to influence real-world adoption of safer chemicals.

615 Boat Antifouling Technology Alternatives Assessment

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Copper is used in antifouling boat paints to prevent marine life from attaching or growing on boat hulls, which results in poor fuel efficiency, less maneuverability, and lower top speeds. In 2007, an Ecology study found high levels of copper in two Puget Sound marinas – most of it coming from antifouling paints. Copper build-up is a concern because of its adverse effects on fish, especially to young salmon. In Washington, fishing accounts for $3.18 billion of economic activity and supports over 17,256 jobs in the state. As a result, the Washington Legislature passed a law that phases out the use of copper-based anti-fouling paints in recreational boats beginning in 2018. Similar regulations are being considered in other states and countries. Problems with antifouling paints are not new. For many years, paints containing mercury or tributyltin were used as anti-fouling paints. Tributyltin was also initially identified as environmentally safe. When concerns arose in the 1970's about the environmental effects of tributyltin, copper became the preferred alternative. Now research has revealed the environmental issues surrounding copper. In the interest of avoiding yet another regrettable substitution—replacing one toxic chemical with another -- the WA Department of Ecology and Northwest Green Chemistry have partnered with industry leaders like the Clean Boating Foundation to evaluate alternatives to copper antifouling boat paint. A variety of anti-fouling technologies are considered including those that are currently on the market and those that are emerging. Some technologies involve substitution of copper with zinc-based coatings. Others involve the synthesis of organic chemicals that do not contain metals and still others involve completely different approaches to anti-fouling such as the use of ultrasound or "slippery" coatings to which organisms cannot attach. The alternatives assessment approach used is based upon the framework developed in the Interstate Chemicals Clearinghouse (IC2) Alternatives Assessment Guide (IC2 Guide). The toxicity of chemicals in each of the alternatives along with other factors inherent in an alternatives assessment such as performance, cost and availability, exposure, etc. are considered as part of the assessment and analysis process. Here, we present our preliminary results.

Toward Sustainable Environmental Quality: Identifying Global Research Needs Through the SETAC Horizon Scanning Project

616 Towards Sustainable Environmental Quality: Overview and Methods of a Novel Effort to Identify Global Research Needs

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The SETAC World Council launched the Global Horizon Scanning and Prioritization Project aimed at identifying geographically specific research needs to address stressor impacts on environmental quality. Priority research questions were solicited from SETAC members and other environmental professionals within the five SETAC geographical units (GU), then synthesized by expert teams of academic, industry and government representatives to form lists of the top research questions that, if answered, would substantially advance our understanding of how a range of environmental stressors (chemical, physical, biological) impact environmental quality in different geographic regions. During 2015, workshops were held for SETAC Africa (in Langebaan, South Africa), SETAC Europe (in Barcelona, Spain), SETAC Latin America (in Buenos Aires, Argentina) and SETAC North America (in Salt Lake City, USA). For SETAC Asia-Pacific, an initial workshop in 2015 focused on Oceania (in Nelson, New Zealand) while a second workshop in Singapore during 2016 focuses on other parts of Asia-Pacific. Each workshop was chaired by SETAC members representing academic, business and government sectors. Questions received were scientific in orientation, and covered diverse aspects of fields related to SETAC's mission to advance sustainable environmental quality. This presentation provides a project overview and methods to identify major research themes emerging within and across SETAC Geographic Units. It is anticipated that outputs from this effort will help increase the quality and relevance of environmental research, decrease scientific uncertainty in assessing and managing environmental risks, and increase the credibility of technical and policy responses to global environmental stressors.

617 Towards Sustainable Environmental Quality: Priority Research Needs for Europe

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The SETAC World Council launched the Global Horizon Scanning and Prioritization Project aimed at identifying geographically specific research needs to address stressor impacts on environmental quality. Priority research questions were solicited from SETAC Europe members and other environmental professionals. A total of 151 questions were received, which were scientific in orientation, and covered diverse aspects of fields related to SETAC's mission to advance sustainable environmental quality. Research questions were initially partitioned among the following breakout themes: 1. Aquatic and terrestrial ecotoxicology: ecology and field; 2. Aquatic and terrestrial ecotoxicology: tiered testing; 3. Analysis, fate and behavior of contaminants; 4. Ecosystem responses and services under multistress: multistress; 5. Ecosystem responses and services under multistress: mixtures; 6. Exposure and effect modeling & predictive toxicology (environment & human); 7. Risk assessment, regulation and public perception: validity of assessment; 8. Risk assessment, regulation and public perception: methods and standards; and 9. Characterization, fate and effects of nanomaterials. During a 2 day workshop in Barcelona, these questions were then synthesized by academic, business and government representatives to identify 22 priority research needs. This presentation provides an overview of major research themes from Europe. Following the workshop these research quesitons were sent to SETAC Europe members, who ranked questions of relative importance. Highly ranked questions ranged from research needed to understand consequences of multiple stressors and chemical mixtures, adverse outcomes across multiple levels of biological organization, and chemical emissions through space and time to development and selection of mechanistic toxicokinetic and toxicodynamic modeling tools and identification protection goals. It is anticipated that outputs from this effort will help increase the quality and relevance of environmental research, decrease scientific uncertainty in assessing and managing environmental risks, and increase the credibility of technical and policy responses to global environmental stressors. SETAC further anticipates these questions will support its Long Range Planning efforts.

618 Towards Sustainable Environmental Quality: Priority Research Questions for Australasia

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Authors: Pettigrove V, Sevicke-Jones G, Manning T, Gaw S, Ataria J, Cameron M, Coates G, Death C, Harford A, Hassell K, Hoak M, Gadd J, Jolley D, Karami A, Kotzakoulakis K, Lim R, McRae N, Metzeling L, Mooney T, Myers J, Pearson A, Sharley D, Sutherland O, Thomas O, Tremblay L, Wood W, Rudd M, Ankley G, Boxall A, Brooks B. The Global Horizon Scanning and Prioritization (GSHP) Project is a SETAC initiative that aims to collect and prioritize the most important future environmental quality research questions as recognized by scientists and engineers from around the globe. We report here the conclusions of the Australasia (AU) workshop held in Nelson, New Zealand in August 2015. Prior to the workshop, SETAC members and other scientists were invited to submit research questions, which in their view, were priority needs to address in the region. 76 questions were received. During the workshop, these questions were partitioned and the top 18 selected. A general question raised was how can ecotoxicology become more relevant to the protection of ecosystems, particularly in terms of making sure ecotoxicology and guidelines are relevant to local issues. Several priority questions deal woth the theme of how widely international ecotoxicological data and databases can be applied to regional ecosystems. It was noted that Australia and New Zealand have many endemic species and there is little information on their sensitivity to contaminants. Many challenges for the development of guidelines were identified. These include the need to develop guidelines for ephemeral water bodies, incorporating information on the effects of short-term exposures to contaminants, plus the use of biomarkers and other sub-lethal responses to stressors and how guidelines can be made to consider mixtures, especially pesticides and veterinary pharmaceuticals. More toxicological data on local species needs to be gathered and incorporated into regional guidelines. Climate change issues were also raised regarding their ecotoxicological implications. One example was considering the effect of predicted water temperature and ocean acidity changes on ecotoxicology of marine environments. An important question raised was how do we incorporate and protect cultural and social values to empowr citizen, especially indigenous engagement into research, management and legislation.

619 Towards Sustainable Environmental Quality: Priority Research Questions for Latin America (LA)

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The Global Horizon Scanning and Prioritization (GSHP) Project is a SETAC initiative that aims to collect and prioritize the most important future environmental quality research questions as recognized by scientists and engineers from around the globe. We report here the conclusions of the Latin America (LA) GSHP Workshop held in Buenos Aires in September 2015. Prior to the workshop, SETAC members and other scientists from LA were asked to submit research questions which, in their view, were priority needs to address in the region. 100 questions were received. These were organized in 6 categories and ranked. The resulting top 20 questions were: Environmental Chemistry: -Adsorption/release of toxic compounds form plastic wastes, -Impacts of pharmaceuticals in aquatic ecosystems, -Quality criteria and limits for environmental legislation. -Quantification of contaminant metabolites and breakdown products in abiotic and biotic matrices. Ecotoxicology: -Climate change influence on fate and effects of pollutants, -Extrapolation of results from regulatory single species toxicity test to local taxa and scenarios, –Understanding the complexity of pesticide toxicology in ecosystems, -Laboratory and field methods and approaches to address ecological complexity. Health, CECs and Environment: -Impacts of nanomaterials on ecosystems and human health, -Algal toxins production and risk for human health. Risk Assessment: -Characterization of individual and combined risks of diffuse chemical contaminants. -Development and refinement of tools for sustainable production and use of chemicals. -Risks characterization of pulp mills effluents for human and the environment. Environmental Management and Policy: -Continuous improvement of the maximum limits of toxicity for agriculture, and domestic and industrial effluents, while maintaining ecosystem services. -Current environmental regulations effectiveness and its implementation and enforcement in LA. -Standardization and harmonization across LA countries. -Effectiveness of protected areas to safeguard biodiversity from pollutants. -Risk management improvement for solid wastes. Spotlight on LA: -Sensitivity information of regional species for better prediction of impacts on local ecosystems. -Impact evaluation of natural resources extraction. -Tools for characterizing and classifying industrial and residential effluents. These questions should be considered as part of a long-term strategic research plans in LA.

620 Towards Sustainable Environmental Quality: Priority Research Questions for Africa

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The Global Horizon Scanning and Prioritization (GSHP) Project is a SETAC initiative that aims to collect and prioritize the most important future environmental quality research questions as recognized by scientists and engineers from around the globe. In this presentation the conclusions derived during the African GSHP Workshop held at Langebaan in October 2015 are presented. SETAC Africa and other scientists in the region were requested to submit the priority research questions. A total of 96 questions were received prior to the workshop. During the workshop these questions were refined and partitioned to select the top 24 questions from six categories: Capacity building in Environmental Toxicology and Chemistry in Africa, Mixtures and multiple stressors, Risk Assessment in the African Context: Policy and Regulations, Contaminants of Emerging Concern, Linking Science and Society and Environmental Chemistry: Analysis, fate and exposure. The prioritized questions included: overcoming the lack of and access to equipment, consumables and facilities to enhance African environmental research output, integrity and intercontinental cooperation; enhancing mentoring programs in increasing or building environmental science research and training capacity; developing innovative cross border collaboration with specialists in various environmental fields to fast track solutions to pressing environmental problems; develop capacity in environmental and health science education, communication and dissemination to raise awareness; cumulative effects of converging agricultural, mining and urbanization on ecosystem and human health; altered risk profiles due to climate change; the risks of multiple stressors to iconic African wildlife, emerging contaminants such as plastics, e-waste, genetically modified crops/organisms and fracking; identification and management of susceptible ecosystems; trans-boundary "superfund sites"; integration of disease and poverty into human health risk assessments following; human health as a result of changes in the chemical use and production patterns due to global changes; indigenous knowledge systems to develop sustainable environmental protection programs in Africa; the effect of trade, trans-boundary movement and quality of products, food and feedstuffs on human and environmental health. There is a realisation that the critical environmental questions need to be addressed in concordance with issues affecting human health.

621 Towards Sustainable Environmental Quality: Priority Research Questions for North America

A. Fairbrother, Exponent, Inc. / EcoSciences; D.C. Muir, Environment and Climate Change Canada / Aquatic Contaminants Research Division; K.R. Solomon, Univ of Guelph / School of Environmental Sciences; J. Denton, RioTinto; J. Apell, MIT / Civil Environmental Engineering; K. Armbrust, Louisiana State Univ / Environmental Sciences School of the Coast and Environment; B. Blalock, Univ Massachusetts Boston / School for the Environment; S.R. Bowman, Michigan Dept of Environmental Quality; L. Campbell, Saint Marys Univ / Dept of Environmental Science; G.P. Cobb, Baylor Univ / Dept of Environmental Science; K.A. Connors, Procter & Gamble / Global Product Stewardship; D.A. Dreier, Univ of Florida / Physiological Sciences; M. Evans, Environment and Climate Change Canada; C. Henry, George Washington Univ; R. Hoke, EI DuPont de Nemours and Company / Haskell Laboratory; M. Houde, Environment and Climate Change Canada / St Lawrence Center; H.D. Sutton, Kennesaw State Univ / Dept of Bio. & Phys. Sciences; S.J. Klaine, Clemson Univ / Clemson Inst of Environmental Toxicology; R. Klaper, Univ of Wisconsin-Milwaukee / School of Freshwater Sciences; S. Kullik, Health Canada / Environmental Impact Initiative; R.P. Lanno, The Ohio State Univ / Dept of Evolution Ecology and Organismal Biology Subsurface Energy Resources Center; C.L. Meyer, Shell Oil Company / Ecotoxicology; A. Ott, Newcastle Univ; M. Ottinger, Univ of Houston / Dept of Animal and Avian Sciences; E. Oziolor, Baylor Univ / Environmental Science; E.J. Petersen, National Inst of Standards and Technology / Biosystems and Biomaterials Division; H.C. Poynton, Univ of Massachusetts, Boston / School for the Environment; P. Rice, USDA-ARS; G. Rodriguez-Fuentes, Universidad Nacional Autonoma de Mexico / Unidad de Química Sisal; A. Samel, DuPont Crop Protection; J. Shaw, Indiana Univ / The School of Public and Environmental Affairs and The Center for Genomics and Bioinformatics; J.A. Steevens, USGS / Environmental Laboratory; T.A. Verslycke, Gradient; D. Vidal-Dorsch, VDA LCS / Toxicology; S.M. Weir, Queens Univ of Charlotte / Biology; P.W. Wilson, Sanofi US, Inc. / Health Safety and Environment; G.T. Ankley, USEPA / National Health and Environmental Effects Research Laboratory; A. Boxall, Univ of York / Environment Dept; M. Rudd, Emory Univ; B.W. Brooks, Baylor Univ / Environmental Health Science Program Dept of Environmental Science

The SETAC World Council launched the Global Horizon Scanning and Prioritization Project aimed at identifying geographically specific research needs to address stressor impacts on environmental quality. Priority research questions were solicited from SETAC North America members, members of the American Chemical Society's (ACS) Environmental Chemistry and Agrochemicals Divisions and other environmental professionals. A total of 310 questions were received, which were scientific in orientation, and covered diverse aspects of fields related to SETAC's mission to advance sustainable environmental quality. Research questions were initially partitioned among the following breakout themes: 1. Ecotoxicology and Applied Ecology: Multigeneration and Field; 2. Environmental Chemistry: Analysis, Fate and Exposure; 3. Regulatory Challenges and Societal Issues; 4. Predictive, Alternative and High-throughput Approaches; 5. Environmental Management: Restoration and Engineering Science; 6. Multiple Stressors (including Mixtures and Climate Change); 7. Eco- and Environmental Toxicology; 8. Sustainability Sciences (including Green Chemistry); and 9. Reducing Uncertainty in Hazard and Risk Assessment. During a 2 day workshop in Salt Lake City, these questions were then synthesized by academic, business and government representatives, including liaisons from the ACS divisions, to identify 40 priority research needs. This presentation provides an overview of major research themes from North America. It is anticipated that outputs from this effort will help increase the quality and relevance of environmental research, decrease scientific uncertainty in assessing and managing environmental risks, and increase the credibility of technical and policy responses to global environmental stressors. SETAC further anticipates these questions will support its Long Range Planning efforts.

622 Towards Sustainable Environmental Quality: Priority Research Needs for Asia

K.M. Leung, The Univ of Hong Kong; J. You, Jinan Univ / School of Environment; C.R. Arias-Barreiro, PETRONAS / Global Toxicology Health Safety Environment; G.T. Ankley, USEPA / National Health and Environmental Effects Research Laboratory; A. Boxall, Univ of York / Environment Dept; M. Rudd, Emory Univ; B.W. Brooks, Baylor Univ / Environmental Health Science Program Dept of Environmental Science

The SETAC World Council launched the Global Horizon Scanning and Prioritization Project aimed at identifying geographically specific research needs to address stressor impacts on environmental quality. Priority research questions were solicited from SETAC Asia-Pacific (sans Australasia, which held a workshop in Nelson NZ) members and other environmental professionals. Quesitons were partitioned among six breakout groups during a 1 day workshop during the SETAC Asia-Pacific meeting in Singapore then synthesized by academic, business and government representatives to identify priority research needs. This presentation provides an overview of major research themes from Asia. It is anticipated that outputs from this effort will help increase the quality and relevance of environmental research, decrease scientific uncertainty in assessing and managing environmental risks, and increase the credibility of technical and policy responses to global environmental stressors. SETAC further anticipates these questions will support its Long Range Planning efforts.

623 Towards Sustainable Environmental Quality: Initial Synthesis of a Novel Effort to Identify Global Research Needs

B.W. Brooks, Baylor Univ / Environmental Health Science Program Dept of Environmental Science; G.T. Ankley, USEPA / National Health and Environmental Effects Research Laboratory; A. Boxall, Univ of York / Environment Dept; M. Rudd, Emory Univ / Dept of Environmental Sciences

The SETAC World Council launched the Global Horizon Scanning and Prioritization Project aimed at identifying geographically specific research needs to address stressor impacts on environmental quality. Priority research questions were solicited from SETAC members and other environmental professionals within the five SETAC geographical units (GU), then synthesized by expert teams of academic, industry and government representatives to form lists of the top research questions that, if answered, would substantially advance our understanding of how a range of environmental stressors (chemical, physical, biological) impact environmental quality in different geographic regions. During 2015, workshops were held for SETAC Africa (in Langebaan, South Africa), SETAC Europe (in Barcelona, Spain), SETAC Latin America (in Buenos Aires, Argentina) and SETAC North America (in Salt Lake City, USA). For SETAC Asia-Pacific, an initial workshop in 2015 focused on Oceania (in Nelson, New Zealand) while a second workshop in Singapore during 2016 focuses on other parts of Asia-Pacific. Each workshop was chaired by SETAC members representing academic, business and government sectors. Questions received were scientific in orientation, and covered diverse aspects of fields related to SETAC's mission to advance sustainable environmental quality. This presentation provides a project overview and synthesis of major research themes emerging within and across SETAC Geographic Units. It is anticipated that outputs from this effort will help increase the quality and relevance of environmental research, decrease scientific uncertainty in assessing and managing environmental risks, and increase the credibility of technical and policy responses to global environmental stressors.

Alternative Approaches to Complex Environmental Challenges – Part 1

624 Assessing and Managing Aquatic Stressors In A Changing Global Environment

P.M. Chapman, Chapema Environmental Strategies Ltd

We are facing a dynamic future in the face of multiple stressors acting individually and in combination: climate change; habitat change/loss; overuse of resources (e.g., overfishing); invasive species; harmful algal blooms/eutrophication; and, chemical contaminants. Respective changes to aquatic environments will be discussed with a particular focus on climate change (e.g., decreased ocean pH), whose effects are expected to be greater in polar and tropical areas than in temperate areas. Risks from climate change and other stressors will be dynamic and changing; historic assessment and management approaches will be inadequate. Wicked problems (non-linear, complex, competing risks and benefits, not easily solvable), which will become increasingly common, will be discussed with examples. The importance of agreed protection goals and considering both the negatives (risks) and the positives (benefits) will be discussed. The talk will conclude with three key questions for researchers and managers will be outlined: determining tipping points; maintaining ecosystem services; and, managing competing ecosystem services.

625 Personal exposure to PAHs near natural gas extraction

L. Paulik, Oregon State Univ / Environmental and Molecular Toxicology; K. Hobbie, ICF / Health Science; D. Rohlman, Oregon State Univ / Environmental Health Sciences Center; R.P. Scott, B.W. Smith, Oregon State Univ / Dept of Environmental and Molecular Toxicology; L. Kincl, Oregon State Univ / College of Public Health and Human Sciences; E.N. Haynes, Univ of Cincinnati / Center for Environmental Genetics; K.A. Anderson, Oregon State Univ / Environmental and Molecular Toxicology

Natural gas extraction (NGE) has expanded rapidly in the United States in the last 15 years. Despite concern about environmental impacts, few studies have directly measured emissions coming from NGE. No study has directly measured personal exposures of people living near NGE. Recent research has suggested that NGE emits polycyclic aromatic hydrocarbons (PAHs) into air. This study used low-density polyethylene passive air samplers to measure PAHs near active NGE wells and sites permitted to host future wells in a rural Ohio community. At each site inner and outer rings of samplers were placed around the well pad or the proposed well pad location. This study also used silicone passive wristband samplers to measure personal PAH exposures of people living near these air sampling sites. Samples were analyzed for 62 PAHs using GC-MS/MS, and isomer ratios were used to identify sources of PAH mixtures. ∑PAH levels in air were significantly higher at sites with active NGE wells than at sites without wells (Wilcoxon rank sum test, $p < 0.01$). Isomer ratios indicated that PAH mixtures at sites with active NGE wells had more petrogenic signatures, while sites without wells had more pyrogenic signatures. This is consistent with NGE well sites being more heavily affected by emissions from within the earth. ∑PAH levels were significantly higher in wristbands from participants who had NGE wells on their home properties than from participants who did not (Wilcoxon rank sum test, $p < 0.005$). Specifically, median ∑PAH was 4.5 times higher in wristbands of participants living within 0.75 km of active NGE wells than in wristbands of participants living farther than 2.0 km from any active NGE wells. There was a significant positive correlation between ∑PAH in participants' wristbands and ∑PAH in air measured at the stationary sampling site nearest to each participant's home (simple linear regression, $p < 0.01$). This work provides further evidence affirming that NGE emits PAHs into air. These findings also suggest that living on a property with an active NGE well may increase personal exposure to PAHs.

626 Risk Communication Lessons from the Lower Duwamish Waterway Fishers Study – One Size Does Not Fit All

S. Replinger, K. Godtfredsen, Windward Environmental LLC; D. Williston, King County / Dept of Natural Resources and Parks; M. McNees, Windward Environmental LLC; K. Pace, Public Health Seattle & King County / Local Hazardous Waste Management Program; S. Reilly, ECOSS; J.H. Stern, Dept of Natural Resources and Parks / Dept of Natural Resources and Parks

As part of the Superfund cleanup process for the Lower Duwamish Waterway (LDW) in Seattle, WA, the Lower Duwamish Waterway Group conducted a fishers study to gather information from people who either harvest or consume seafood from the LDW. The objective of this study was to inform the development and improve the effectiveness of institutional controls (ICs) related to the consumption of resident seafood. ICs will be needed at the site both during and following cleanup. During cleanup, tissue concentrations are expected to be elevated relative to existing conditions as a result of dredging, and following cleanup, tissue concentrations are expected to re-equilibrate at concentrations above human health risk thresholds. The fishers study included two primary components: 400 quantitative surveys were completed in 7 languages with fishers of more than 20 ethnicities as part of a year-long effort to survey fishers along the river and 11 qualitative key informant interviews were completed with a total of 22 community members that allowed for more in-depth discussions with people who fish on the LDW and people who are preparers or consumers of LDW seafood. Among the key findings of the study is a better understanding of who is fishing on the river, who is consuming LDW resident seafood, and how fishers and consumers of LDW seafood prefer to receive health information about the Duwamish. In addition, the study provided insight into the risk models of Duwamish fishers. Specifically, risk associated with the consumption of resident seafood is primarily perceived through the fisher's perception of the cleanliness of the water, visual inspection of the seafood, experience (or lack thereof) of acute sickness, and word of mouth. This and other information learned over the course of the study will be used to shape future communication strategies about long-term health risks from eating contaminated seafood from the Superfund site. The results of the study show that differences in age, gender, cultural background, and other factors mean that a one-size-fits-all approach to risk communication will not be successful, but rather risk communication must include a variety of methods (e.g., interactive community-based outreach efforts) tailored to the needs of the community.

627 Evaluating Toxicity-Based Injury Thresholds for Contaminants at the Hanford Nuclear Site Using Multiple Empirical Approaches

T. Baker, NOAA / Office of Response Restoration; B. de Jourdan, Oregon State Univ / Environmental and Molecular Toxicology; S. James, US Fish and Wildlife Service

A group of eight State and Federal agencies and Tribes are participating in a Natural Resource Damage Assessment (NRDA) at the Hanford Nuclear Site in Eastern Washington. The Site has more than 40 contaminants of potential concern (COPCs) within a large pollution footprint, including portions of the Columbia River. Because of Hanford's size, complex place in U.S. history, current cleanup status, and long-term nature of contamination, information relevant to injury threshold-setting is voluminous and of highly variable quality and degree of completeness. Open access and transparency during the entire threshold evaluation was non-negotiable among Trustees. All of these factors and the legal overlay of the NRDA process made consensus-based decision-making in traditional ecotoxicity-based frameworks challenging. We successfully used an iterative, Workshop-based process to first frame the issue of injury thresholds to a large group of NRDA stakeholders, and then reach interim understandings about the range of injury threshold values in soils, surface waters, and sediments that should be considered for taking the next step in the injury assessment – quantifying injuries to resources and ecological services. Over several years, we collaboratively drafted technical memos focused on understanding ecological effects from a single contaminant and maintained all analyses as open access. For many COPCs, we used results from multiple empirical approaches to inform our NRDA evaluation, notably a soil Predicted No-Effect Concentrations (PNEC) calculator, Biotic Ligand Models, Consensus-Based Sediment Quality Guidelines, and Equilibrium Partitioning approaches. We also undertook an extensive evaluation of background contaminant concentrations, considered existing screening-level benchmarks and ecological injury thresholds, criteria, standards, and guidance values, reviewed peer-reviewed literature on sub-lethal endpoints and mixture toxicity, and cross-walked our preliminary findings to Site-specific ecological risk analyses and laboratory toxicity tests. At Hanford, an iterative and collaborative Workshop approach with completely open data access and well-developed memoranda has led to significant progress.

628 Hormone receptor bioanalytical assays as indicators of endocrine disrupting potential: Case studies from the field and laboratory

J.H. Bisesi, S. Robinson, Univ of Florida / Envirionmental and Global Health; L. Ferguson, Duke Univ / Dept of Civil and Environmental Engineering; N.D. Denslow, Univ of Florida / Physiological Sciences; T. Sabo-Attwood, Univ of Florida / Environmental Global Health

Aquatic environments are often inundated with complex mixtures of contaminates with endocrine active modes of action. Bioassays to assess the toxicity of point source outflows and surface waters can often be expensive and time consuming; often requiring follow up testing to determine specific mechanisms of toxicity. High throughput bioanalytical assays using hormone receptors have emerged as a technique to identify the cumulative impacts of complex mixtures on specific endocrine modes of action. Through collaborative efforts we have developed a fluorescence based estrogen receptor binding assay as well as a luciferase based estrogen receptor transactivation assay. Both assays are plate based, high throughput assays with great sensitivity. This presentation will focus on potential uses of such assays with examples from the lab and field including: (1) Identification of estrogenicity of emerging contaminants. As new chemicals are developed and released in our aquatic environments it is important to understand their mode of action in aquatic species. Using these assays we can quickly assess a chemicals ability to bind and activate or deactivate the estrogen receptor. (2) Assessing the estrogenic potential of contaminated field sites. Following the collection of water samples and subsequent extractions from field sites in the US as well as Haiti, we have successfully used these assays to identify sites of potential concern due to elevated estrogenic activity. (3) Determining how interactions of nanomaterials and estrogens impact downstream responses. Emerging contaminants like nanomaterials have the potential to interact with estrogenic compounds already present in our environment. Using these assays we have shown the single walled carbon nanotubes have the potential to sorb estrogens and decrease estrogen receptor binding and activation. These examples demonstrate the versatile nature of in vitro hormone receptor assays for assessing the impacts of complex mixtures of aquatic contaminants. Results from these assays can be used to prioritize sample testing, which has been recommended under the adverse outcome pathway paradigm.

629 The Differential Binding of New River Wastewater Effluent EDCs to the New TriFishER Assay for Measuring Estrogenicity

B. Tate, S. Tuberty, Appalachian State Univ / Biology; B. Hawkins, North Carolina State Univ / Dept of Biological Sciences

Endocrine disrupting compounds (EDCs) have been identified in aquatic environments, are known to mimic natural estrogens (e.g.17ß-estradiol), and disturb reproduction and development of aquatic wildlife. One important mechanism of EDC action is modulation of activation of estrogen receptors (ERs). The identification of specific EDCs that interact with ERs is a complex issue due to the vast diversity of endocrine disrupting chemicals found in the environment. Even more uncertain is the number of the ~70,000 chemicals not yet tested for EDC activity. Therefore, it remains unknown if these unidentified chemicals contribute to the combined effects of EDCs. Although vertebrates possess multiple ER subtypes with different ligand binding and transactivation properties, most screening is done using a single ER subtype, the human ERα. This approach may result in an underreporting of estrogenic contamination, particularly for non-mammalian aquatic species. Teleost fish express three ER subtypes (ERa, ERba, and ERbb), with ligand binding profiles distinct from each other and mammalian ERs (ERa and ERb). We report here a new validated assay called the TriFishER that applies all three fish estrogen receptors (α,β_a & β_b). In addition to being more environmentally relevant, the TrifishER assay is economical, more time efficient (~2 days), while maintaining sensitivity in the nanogram/liter range, making it an important tool in determining aquatic EDC binding potential. The Trifisher assay is a proven high throughput competitive binding assay that uses an E. coli expression vector transformed with each of the three acER (Atlantic Croaker estrogen receptor) ligand binding domains. Each class of compound binds differently to each receptor giving the compounds a unique binding profile (i.e. fingerprint), which can then be used identify EDC activity in the environment. To test this hypothesis the Trifisher assay has been used with environmental samples from communities that differ in their average population age along the New River corridor of NW NC. The TriFISHer assay is not able to detect what type of estrogenic compound is present in solution or the precise concentrations of individual estrogenic compounds present in the solution. The TriFISHer assay will also not be able to predict what genes are activated after EDCs bind to the receptors.

630 Riparian Spiders: A valuable tool in ecotoxicological studies

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A major first tier division for most environmental studies is whether exposure is occurring in the aquatic or terrestrial ecosystem. However, some of the most complex scenarios occur when contaminants and/or organisms are exchanged between linked aquatic-terrestrial systems (typically in the riparian zone). Historically, ecotoxicological studies operated under the assumption that once a contaminant enters the aquatic ecosystem it is rendered an aquatic concern only, and the export to the terrestrial ecosystem is insignificant. Early interchange studies highlighted the importance of cross-system subsidies and contaminant transport typically in the form of emerging aquatic insects or salmon. More recently, riparian spiders have gained traction as a valuable tool in this field of study. These abundant and widespread secondary/tertiary consumers feed on aquatic emergent insects and can act as a contaminant bridge to the terrestrial food-chain. They possess life history characteristics that make them attractive as potential model organisms (e.g., ubiquitous distribution, small home ranges, and short life spans). Progressively, more studies are using/studying riparian spiders to investigate a variety of research directions, including contaminant export, food chain dynamics, and contaminant monitoring. In this presentation a chronological overview of key studies utilizing riparian spiders as well as ongoing studies will be discussed.

631 Efficacy & residual toxicity of a NaOH-based ballast water treatment system for freshwater bulk freighters: shipboard trials

A.A. Elskus, USGS / Ecosystems; D.A. Wright, C.L. Mitchelmore, Environmental Research Services; N. Welschmeyer, Moss Landing Marine Laboratories; C.J. Flynn, Glosten Associates; J.W. Henquinet, Henquinet Consulting; P. Pelletreau, American Steamship Company; B.J. Watten, USGS / SO Conte Anadromous Fish Research Laboratory Leetown Science Center

The ballast water (BW) of freshwater freighters is an important pathway of secondary spread of nonnative species in the Great Lakes. However developing BW treatment systems (BWTS) that significantly reduce the viability of BW organisms yet retain little to no residual toxicity upon release pose a significant environmental challenge and there are currently no type-approved BW treatment systems (BWTS) for freshwater ships. The efficacy and residual toxicity of a novel sodium hydroxide (NaOH)-based BWTS were tested aboard the Great Lakes bulk carrier M/V American Spirit during 4 trials conducted in July & September 2015. BW was treated

by adding NaOH to elevate pH to 11.5 or 11.7 for 48 h, then reduced to pH < 9 before discharge by sparging with carbon dioxide gas. The density of organisms after treatment met the 2013 Vessel General Permit discharge standards for all 3 organism size classes (< 10 mm, ≥ 10 mm to < 50 mm, > 50 mm) in both September trials, and for all organism classes in July trials with one exception. To provide additional data, procedures not in the current regulatory standards were added. These included evaluation of total coliform and total heterotrophic bacteria (< 10 mm) which were reduced > 96% at both pH levels relative to intake densities. Additional tests for phytoplankton and protists using adenosine triphosphate concentrations and fluorescein diacetate tagging demonstrated dramatic reductions in the living biomass of these organisms (≥ 10 mm to < 50 mm) at both pH levels. A 7d whole effluent toxicity test indicated NaOH-BWTS discharge water at the highest level tested, pH 11.7, met the vessel general permit daily maximum for residual toxicity. In addition, testing with the 24 h, dinoflagellate-based QwikLite 200 Biosensor System detected no residual toxicity. This BWTS has the potential to become a successful method for treating large volumes of BW released into freshwater systems.

Canadian Oil Sands: Advancing Science in Chemical and Toxicological Characterization, Reclamation and Monitoring – Part 2

632 Thia-arenes and Aza-arenes in Samples from the Oil Sands Area of Alberta

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The Athabasca Oil Sands (AOS) deposits located in Northern Alberta, Canada are considered the world's third largest oil reserve (OPEC, 2014). Long-term data sets on major contaminants, such as polycyclic aromatic compounds (PACs), have been gathered in the past few years as part of the Alberta-Canada Joint Oil Sands Monitoring (JOSM) program, initiated in April 2012. However, most efforts and monitoring plans have focused on a limited list of PACs, which are formed by the incomplete combustion of organic matter during both natural and anthropogenic processes. PACs in the AOS area can be emitted from a large list of potential sources that include natural erosion of geological formations, forest fires, bitumen upgrading, diesel combustion and airborne dust from mining operations. Therefore, samples collected in the AOS area can potentially contain a complex mixture of PACs, which can also include sulfur and nitrogen containing heterocyclic compounds (thia-arenes and aza-arenes). This study was designed to explore PAC isomers currently not being monitored in snowpack samples, lake sediment samples and passive air samples collected at varying distances from the main AOS developments during 2012-2013. All samples were analyzed using two-dimensional gas chromatography with time-of-flight mass spectrometry (GC×GC/ToF-MS), equipped with a liquid-crystalline column in the first dimension and a nano-stationary phase column in the second dimension. Thia-arenes and aza-arenes were identified in all samples collected and classified into isomeric groups, based on their mass spectra and their location in the 2D-chromatogram. Relative concentrations of thia-arenes and aza-arenes decreased with distance from the main developments, and with increasing depth of lake sediments. Additionally, a delayed petcoke sample from local sources, extracted with dichloromethane and methanol, was also analyzed. All thia-arenes and aza-arenes found in the snowpack, lake sediments and air samples, were also found in the delayed petcoke extract. The distribution of thia-arenes and aza-arenes in the petcoke extract was similar to those observed in samples collected within 20 km of the major developments. Therefore, thia-arenes and aza-arenes identified have the potential to be used as source indicators for future research in the area.

633 Developmental and reproductive effects of adult & larval wood frogs exposed to naphthenic acids from commercial sources & oil sands process water

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Naphthenic acids (NAs) are naturally found in the bitumen deposits in Canada's oil sands region, and through the extraction process, can become concentrated in oil sands process-affected water (OSPW). OSPW is currently stored in tailings containment facilities on oil sands sites as they are not currently regulated for discharge. As part of the industry's reclamation plan, strategies are looking at whether or not OSPW can be treated and safely discharged back into the environment. Therefore, as a part of these efforts, it is important to understand the effects of NAs and OSPW on the health of amphibians at different stages of development. Adult Wood frogs (L. sylvaticus) were caught near Bishops Mills, Ontario, Canada and bred in lab. Egg masses were allowed to develop until Gosner stage 8-10 (embryonic) and 20-21 (hatchling) before tadpoles were exposed to commercial NAs (Merichem Co.; 0-196 mg L^{-1}) and NAs extracted from OSPW (0-190.5 mg L^{-1}). In both studies, tadpoles were photographed 96hr post exposure to determine morphometric parameters, abnormalities and survival rate. Embryonic wood frogs exposed to 8-12 mg L^{-1} of Merichem NAs were significantly smaller in size, had reduced pigmentation and showed compromised structural integrity of the gut; particularly uncoiling of the intestines and thickening of the intestinal lumen. Surprisingly, hatchlings exposed to the same NA concentrations exhibited few alterations until higher NA concentrations (48 mg L^{-1}), suggesting a critical window of exposure for this species. When exposed to NAs extracted from OSPW, hatchling wood frogs showed similar developmental impacts as with Merichem exposure, however not until concentrations approached 80 mg L^{-1}. Adult wood frogs were also exposed to NAs extracted from raw OSPW during spawning. Exposure to 3, 10 and 30 mg L^{-1} disrupted spawning behavior with reduced occurrence and time in amplexus. All eggs laid by frogs exposed to 3, 10 and 30 mg L^{-1} were abnormal in development, suggesting NAs from OSPW could be reducing fecundity. Efforts are still underway to find causative agents in the very complex mixture of NAs and to understand whether or not effects observed in the lab would occur with NAs exposures in the wild. Nevertheless, these results help further our understanding of the toxicological properties of NAs and their impact on a key bioindicator species.

634 Naphthenic acids derived from oil sands processed waters are respiratory uncouplers

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The extraction of bitumen from oil sands via the surface mining produces large volumes of waste water. Naphthenic acids, a complex mixture of carboxylic acids, are a dominant group of compounds present in the oil sands process affected water. Naphthenic acids have been shown to acutely lethal to fishes as well as cause a variety of sublethal effects, including: endocrine disruption, developmental abnormalities, and inhibition of growth. No over-arching mechanism of action for these acids has been found. It was hypothesized that naphthenic acids would act as respiratory inhibitors due to their carboxylic acid moiety, and based on observations from other carboxylic acids that have been observed to disrupt cellular energetics. It was predicted that NAs will inhibit the electron transport chain and uncouple oxidative phosphorylation and that these effects will be mediated by oxidative stress. To test this hypothesis, naphthenic acids were extracted from 17-year-old tailings from Syncrude Canada using acid precipitation followed by DEAE cellulose cleanup methods, DCM liquid-liquid extraction, and a subsequent precipitation under acidic conditions. The precipitate was washed with distilled water and freeze dried in order to generate a solid material. Naphthenic acids were characterized using HRMS and found to be greater than 95% pure based on the negative ion spectra. Mitochondria were isolated from rainbow trout liver cells using differential centrifugation and cellular respiration parameters were tested using an Oroboros respirometry system. Mitochondrial redox state and membrane potential were evaluated using real-time flow cytometry in combination with the fluorescent dyes, DCFDA and JC-1. Results have shown inhibition of cellular respiration following naphthenic acid exposure at concentrations above 120 mg/L. Naphthenic acids specifically caused a reduction in stage 4 respiration. Results will be discussed with regards to the environmental relevance of the dose-effect relationship observed here.

635 Effects of elevated salinity and dissolved organic matter in surface water from an oil sands end-pit lake on the toxicity of metals to zooplankton (Ceriodaphnia dubia)

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In order to manage growing inventories of fluid fine tailings (FFT) and oil sands process-affected water (OSPW), Alberta oil sands mine operators have proposed end-pit lakes (EPLs) as a method for the long-term storage and reclamation of tailings. In December of 2012, Syncrude began the industry's first large-scale demonstration of EPLs with the creation of Base Mine Lake (BML) – an artificial lake containing FFT capped with OSPW and fresh water. However, tailings contain a complex mixture of dissolved organics, salts and metals which have adverse effects on phytoplankton and zooplankton – aquatic organisms essential for early ecosystem development. The initial elevated salinity of BML decreased rapidly during 2013 and 2014, but has since slowed. The chloride concentration (410 mg/L) is now within a tolerable range for most freshwater organisms but will still cause stress. From October 2014 to August 2015, most dissolved metal concentrations decreased an average of 60%; Cu and Zn were the only metals which increased (>300% and >200%, respectively). As of August 2015, Cu and Hg are the only metals which still exceed Canadian Water Quality Guidelines, although As, Cd and Cr all exceeded in 2014. Despite these decreases, some metals remain highly elevated above natural background levels (Mo is 45x higher in BML than in the Athabasca River). Comparisons to chronic toxicity values for model phytoplankton and zooplankton species (Raphidocelis subcapitata and Ceriodaphnia dubia, respectively) yield hazard quotients >0.05 for Cu, Ni and U only. The vast majority of all other dissolved metals now have chronic toxicity hazard quotients < 0.01 for both species and are no longer of any concern. As a result, current work is focused on characterizing the potentially attenuating effects of the elevated BML salinity, alkalinity and dissolved organic carbon on the toxicity of Cu, Mo, Ni, and U to these initial aquatic colonizers. Our research will allow Syncrude to more adequately evaluate the efficacy and sustainability of EPLs as a method for long-term oil sands reclamation. This is crucial, as the results of the BML demonstration will have a profound impact on the future development of oil sands within Canada and around the world.

636 The impact of raw and ozonated oil sands process water exposure on prey capture and facial morphometrics in zebrafish larvae

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In the oil sands mining industry, oil sands process water (OSPW) is stored in tailings containment facilities to ensure it can be recycled for bitumen extraction, process cooling and hydro-transport of materials (e.g., tailings slurries). As part of the industry's reclamation plan, tailings containment facilities will eventually be developed into terrestrial or aquatic habitat that can sustain functions similar to natural habitat in the region. The effectiveness of ozone treatment in increasing OSPW quality is under study to determine the suitability of this treatment method. The impact of OSPW exposure on early developmental stages of fish has been well studied, however, the sublethal impacts of raw and ozonated OSPW exposure in later developmental stages have yet to be determined. During the growth and development of juvenile fish, feeding efficiency is ecologically important. Cardiac function and craniofacial structure can have a large impact on the success and speed of prey capture, which can translate into differences in larval growth, maturation and reproductive success. In this study we examined the effect of embryological exposure to raw and ozonated OSPW from two different mine sites on the craniofacial morphology, cardiac function and prey capture success of zebrafish larvae. The study of more ecologically relevant endpoints, such as prey capture and feeding behavior, on well characterized teleost model species, such as the zebrafish, will allow a better understanding of potential impacts of OSPW on fish species and the suitability of ozone for treatment of OSPW.

637 Long-term effects of oil sands process-affected water (OSPW) on growth, reproduction, and energy reserve of Daphnia magna

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Determining the potential effects of oil sands process-affected water (OSPW) on aquatic ecosystems is of main concern to oil sands companies and legislators concerned about the reclamation of mining sites. In the present study, the effect of OSPW on energy reserve, growth, and reproduction of Daphnia magna was investigated. One-week old D. magna were exposed to 1 and 10% OSPW and culture water as control, for 10 days. The neonates produced were removed and counted throughout the exposure. The length and dry weight of both the adult and neonate daphniids was measured at the end of exposure. To investigate the energy reserve in exposed daphniids, total hydrocarbon, protein, and lipid was measured. The number and weight of neonates in 1% group was not different from the control, but the daphniids exposed to 10% OSPW produced fewer and smaller neonates. The weight of daphniids in 10% OSPW was lower than the control, but not different from control in the 1% group. Neither of the treatments had an effect on daphniid length. Energy reserves of daphniids exposed to OSPW is lower than the control group. The results of the present study suggest that exposure to OSPW reduces the fitness of individual D. magna that may impose ecological pressure on D. magna population.

638 Identification of chemical classes contributing to the toxicity of oil sands process affected water

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Oil Sands process-affected water (OSPW) produced during extraction of bitumen in the surface-mining oil sands industry in Alberta, Canada, can be acutely and chronically toxic to aquatic organisms. It is known that organic compounds that are dissolved in OSPW are responsible for most toxic effects. Here, we present an overview of a four year project with the purpose of identifying chemical classes contributing to the toxicity of OSPW. By use of best available analytical technology, linear ion trap (orbitrap) ultrahigh resolution mass spectrometry operated in positive and negative electrospray ionization modes ($^{+/-}$), and an effects- directed analysis approach, three rounds of sequential fractionation and acute toxicity testing were completed. From this work, two refined fractions of dissolved organic chemicals in OSPW (F3-NE2a and F3-NE2b) were produced and were acutely toxic to exposed embryos of Fathead minnow (Pimephales promelas) and by use of the Microtox® test (Bacterium; Vibrio fisheri).

To support this work, an acute aquatic toxicity model was developed to predict the toxicity of samples of OSPW to embryos of Fathead minnow to further investigate the contribution of chemical classes in the mixture. For model development, a narcosis mode of action was assumed, and the target lipid model of Di Toro et al., (2000) was applied for toxicity predictions by use of measured bioaccumulation estimates of the individual accurate masses, either octanol-water (D_{OW}) or membrane-water (D_{MW}) partition coefficients measured at a pH of 7.4. This work highlighted the contribution of the chemical classes SO^+, SO_2^-, $O_2^{+/-}$ and NO^+ to the acute toxicity of OSPW and demonstrated that an explicit assessment of chemical accumulation into polar lipids by use of D_{MW} improves toxicity predictions. Chronic toxicity of the tertiary fractions (F3-NE2a and F3-NE2b) was investigated by use of the USEPA 21-day Fathead minnow reproduction bioassay to assess effects of dissolved organic chemicals in OSPW on the endocrine system of Fathead minnows exposed under laboratory conditions. Endocrine disrupting effects were assessed by use of various reproductive measurements and morphological, histopathological, and molecular/biochemical responses of males and females. This work has highlighted the contribution of novel chemical classes to the acute and chronic toxicity of OSPW and will aide in the development of reclamation and monitoring programs in the oil sands region of Alberta, Canada.

639 Bioassay-directed fractionation of bitumen-influenced groundwaters from the Athabasca oil sands region

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Much of the oil sand deposit in the area surrounding Ft. McMurray, AB is near-surface, enabling cost-effective surface mining operations in this region. Ground and surface waters can traverse the deposit, possibly mobilizing water-soluble bitumen derived chemicals into the Athabasca River watershed. In addition to this input, recent research has identified oil sands process-affected water (OSPW) contamination of groundwater systems surrounding tailings ponds. While chemical profiles are beginning to be described for these natural and anthropogenic bitumen-influenced groundwaters, little is known regarding their toxicity and the identities of the toxicologically relevant components. In the current study, a preparative fractionation protocol was applied to a sample set of four groundwaters (>100L each), previously identified as containing either natural bitumen or OSPW contamination mixed with natural bitumen. The fractionation method allowed for the isolation of individual mixtures of neutral organics, polar organics, and inorganics (metals and salts), generating five fractions in total. The resulting fractions were assessed using a suite of invertebrate bioassays (in complement to fish bioassays presented separately) for acute toxicity profiles. Assays with Hyalella sp., Daphnia sp., freshwater mussel glochidia and Microtox indicated differences in the observed toxicity between species, between different fractions of the same source and also between sources when comparing the same fractions and species. This work will guide more detailed fractionation studies designed to identify the compound classes of interest within the soluble organic mixtures of bitumen-influenced waters. This information is critical for the development of water monitoring programs in the oil sands region.

Epigenetics and Environmental Exposures: Mechanisms and Effects from Invertebrates to Fishes

640 Role of DNA methylation in AHR-mediated toxicity of PAHs in chicken embryo

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DNA methylation is an epigenetic mark that plays an important role in regulating gene expression. As with other epigenetic marks, patterns of DNA methylation are sensitive to environmental stressors (e.g. contaminants) and have the potential to be heritable (i.e. faithfully copied as cells divide, persisting beyond initial exposure). Here, we assess the role of DNA methylation in aryl hydrocarbon receptor (AHR) mediated toxicity of polycyclic aromatic hydrocarbons (PAHs) in the developing chicken embryo. Graded concentrations of the PAH, benzo[k]fluoranthene (BkF), were injected into the air cell of fertilized unincubated chicken eggs on embryonic day 0 (ED0). Liver tissue was sampled at several timepoints throughout development – ED7, ED10, ED19, and two days post-hatch (D2) – in order to assess BkF-associated patterns of cytochrome P4501A (CYP1A) mRNA expression and DNA methylation. Induction of CYP1A mRNA isoforms CYP1A4 (23-fold) and CYP1A5 (15-fold) was first observed starting on ED10, several days after the start of organogenesis. The transcriptional response was transient. By ED19, the signal was no longer present, a finding that corresponds well with previously published data suggesting that the developing chicken embryo rapidly metabolizes PAHs. In contrast, BkF-associated increases in DNA methylation at key regulatory sequences within the CYP1A4/5 shared promoter persisted through ED19 and were also detected in the hatched chick. We investigate how these persistent epigenetic marks may affect subsequent exposures to AHR ligands using a chicken embryo hepatocyte model. This work begins to explore linkages between early-life experiences in the embryo and later-life responses to PAHs.

641 Multi generational effects of pharmaceutical exposure in zebrafish (Danio rerio)

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Carbamazepine and gemfibrozil are two pharmaceutical contaminants frequently detected in wastewater effluent and surface waters. They are characterized by continual release, long half lives in water, and bioaccumulation with uncertain metabolism rates in fish. Carbamazepine and gemfibrozil are reported to negatively affect male reproduction in mammals and fish. This study investigated the hypothesis that carbamazepine and/or gemfibrozil act as endocrine disruptors via anti androgenic effects, that direct exposure to either contaminant can adversely affect parental male reproductive physiology, and these impacts may be transmitted to unexposed offspring. We exposed adult zebrafish to 10 μgL^{-1} of either carbamazepine or gemfibrozil for 6 weeks and analyzed a range of male reproductive indices, which were also investigated in unexposed F_1 and F_2 offspring to assess multi generational impacts. We observed that exposure to carbamazepine and gemfibrozil caused a decline in breeding success and mean embryo production in F_0 parents. Exposure also resulted in a reduction in whole body, plasma and testicular 11-ketotestosterone (11KT); and altered male courtship, aggression and sperm morphology. The F_1 offspring were raised in clean water from seven distinct lineages where only a single parent or both parents were exposed to either carbamazepine or gemfibrozil. F_1 offspring from exposed paternal lineages had reduced breeding success and mean embryo production as well as altered male courtship, sperm velocity, sperm morphology, and whole body 11KT. Similar findings were recorded for F_2 males. The F_3 offsprings are currently being reared. Our results indicate sub-lethal toxic effects are transferred to unexposed male offspring via parental exposure alone. Preliminary findings suggest carbamazepine and gemfibrozil may act as anti androgens in fish and that exposure to these pharmaceuticals may reduce male reproductive fitness.

642 Assessing benzo[a]pyrene-mediated effects on the zebrafish transcriptome and promoter methylation to explain developmental adverse outcomes

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Benzo[a]pyrene (BaP) is a well-known but mechanistically complicated carcinogen, reproductive and developmental toxicant. In zebrafish (Danio rerio), waterborne or dietary BaP/PAH exposure causes developmental adverse outcomes including growth reduction, neonatal death, cardiac dysfunction, skeletal abnormalities, craniofacial and fin deformities. In an effort to mechanistically explain these toxicities, we performed genome-wide transcriptional analysis and discovered differential gene expression and exon usage in zebrafish at two important developmental stages. Additionally, we measured both global and gene specific promoter methylation by multiplex deep sequencing of 21 genes known for their role in human diseases. Consistent with previous findings on the mechanisms of BaP toxicity, we found that BaP impaired canonical pathways including xenobiotic metabolism signaling, Nrf2-mediated oxidative stress response, glutathione-mediated detoxification, AHR signaling, and estrogen biosynthesis pathways. Novel differentially regulated and disease-related pathways included Notch, melatonin and ApoE pathways. Alternatively spliced myosin and collagen genes may be involved in BaP-induced growth deficits. In BaP-exposed 96 hr post fertilization larvae, global DNA methylation was significantly decreased, and ten genes (6 hyper- and 4 hypomethylated) showed >10% change in CG methylation. Collectively, these molecular changes can be used to predict immediate, long-term, and multigenerational adverse consequences and to develop an AOP for BaP-mediated developmental toxicity. Supported by NIEHS R21ES019940.

643 Differential DNA Methylation in F2 Generation Testicular Cells Caused by Embryonic Exposure to Bisphenol A at F0 Generation

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Bisphenol A (BPA) is a compound used primarily to manufacture polycarbonate plastics and epoxy resins. It is also an additive in other products. Due to extensive use of BPA in commercial products, the threat to human and wildlife health posed by BPA-containing waste in the environment is a potential concern. We have previously observed transgenerational reproductive defects in unexposed F2 and F3 offspring of Japanese medaka (Oryzias latipes) caused by embryonic exposure of F0 offspring to BPA (Bhandari et al., 2015, Scientific Reports 4: 903). Here we report molecular changes, specifically transcript abundance and DNA methylation of select promoters, in the testis of F0 fish (directly exposed as embryos) as well as in the F2 fish that had transgenerational phenotypes of impaired fertilization capacity and increased embryo mortality. BPA induced subtle changes in DNA methyltransferase enzyme expression in germ cells of the F0 adults exposed during embryonic development, and expression of Dnmt genes (Dnmt1, Dnmt3aa, Dnmt3bb) increased 2- to 10-fold in germ cells of the F2 males, accompanied by 2.5-fold increase in global DNA methylation. DNA methylation of the estrogen receptor promoter was significantly reduced in germ cells of F0 but was not altered in F2 germ cells. Elevated DNA methylation levels were maintained at the CpG island of androgen receptor (AR) core promoter of both testicular germ cells and somatic cells in the F2 generation. As expected, the expression of the AR gene in testicular somatic cells was significantly decreased, which confirms an inverse relationship between DNA methylation and gene expression. Together, these findings provide insights into transgenerational inheritance of BPA-induced epigenetic marks by germ cells and soma at the F2 generation.

644 Epigenetic mechanisms mediating responses to Florida Red Tides in the Eastern oyster Crassostrea virginica

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The study of the epigenetic mechanisms mediating exposure-response relationships constitutes the basis for environmental epigenetic analyses, providing information about how different environmental factors influence phenotypic variation. Marine invertebrates are emerging model systems in such studies and, among them, molluscs are remarkable candidates due to their ubiquitous distribution, easy accessibility and diverse lifestyles. However, knowledge about epigenetic mechanisms and their role during environmental acclimatory and adaptative responses is still scarce in these organisms. One of the most important sources of stress in the oceans is the occurrence of Harmful Algal Blooms (HABs), massive algal proliferation episodes where large amounts of harmful biotoxins are produced. Among them, brevetoxins (PbTx) represent the most predominant neurotoxic shellfish poisoning biotoxins in the coasts of Florida. In the present work we have simulated HAB episodes in the lab in order to analyze the epigenetic responses elicited by PbTx exposure in Eastern oysters (Crasostrea virginica). For that purpose, oysters were exposed to increasing concentrations of the PbTx-producing dinoflagellate Karenia brevis. After the simulation, histone variant expression and post-translational modifications (PTMs), as well as DNA methylation were compared between different oysters at different time points. Quantitative gene expression analysis of different histone variant genes did not show any significant differences between different time points, however western blot analysis showed that the amount of PbTx does correlate with the phosphorylation levels of H2A.X, a histone variant known for its involvement in the repair of DNA double stranded breaks. Similarly, oysters exposed to higher concentrations of PbTx showed modifications in global DNA methylation patterns, which may favor genomic stability when they are exposed to the toxin. Overall, the dynamic changes observed in PTMs and global methylation levels are consistent with the role of epigenetic mechanisms in the responses of oysters to marine toxins. Such results set a framework for future studies aimed at developing new markers of marine pollution.

645 DNA methylation reprogramming during development in the self-fertilizing mangrove rivulus and its sensitivity to environmental pollutants

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The mangrove rivulus, Kryptolebias marmoratus, is a small fish native from the mangrove ecosystems of Florida, Bahamas and Central America. It presents a great capacity to withstand the constantly fluctuating environmental conditions that define mangrove ecosystems, and is characterized by a high level of phenotypic plasticity. In natural populations, hermaphrodites coexist with a low proportion of males (androdioecy) and it displays the unique ability for a vertebrate of self-fertilization. Selfing has resulted in populations composed of distinct isogenic strains with a variety of identifiable phenotypes. Using rivulus to study phenotypic plasticity permits the construction of "true" reaction norms by reducing genetic noise within lineages. As a new biological model, molecular mechanisms controlling the key transitions during its life history remain largely unknown. Among them, epigenetic modifications such as DNA methylation have important regulatory functions controlling gene expression, and thus the phenotype. Here, LUminometric Methylation Assay (LUMA) was used to explore the dynamic of global DNA methylation during development, in juveniles and in diverse adult tissues. Significant differences between hermaphrodite ovotestes and male testes were observed (87.2% and 79.6%, respectively). After fertilization, a decrease in DNA methylation occurred from 27.8% in fertilized eggs to 15.8% in gastrula, immediately followed

by an increase and re-establishment of the adult pattern by the stage 26 (liver formation) (70.0%). In addition, characterization of genes coding for DNA-methyltransferase enzymes (DNMT1, DNMT3A and DNMT3B) suggests evolutionary conservation of this gene family. Together these results provide evidence of an original reprogramming pattern of DNA methylation among vertebrates, with a long apparent zygotic transition. To determine how sensitive to environmental stress the reprogramming period is, impacts of different contaminants (EE2, Cu and oil-spill pollutants) on DNA methylation during the zygotic transition is being investigated. Altogether we hypothesize that DNA methylation, and more generally epigenetic mechanisms, may have a crucial role in adaptive evolution of rivulus. Epigenetic mechanisms might provide the plasticity required to deal with myriad environmental challenges, which makes mangrove rivulus a promising new vertebrate model in ecotoxicological epigenetics.

646 Multiple stressors over multiple generations: assessing the combined risk of endocrine disruptors and climate change in an estuarine fish

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Understanding anthropogenic impacts such as climate change and pollution on aquatic ecosystems is critical for preserving biodiversity and maintaining water quality. The pyrethroid pesticide bifenthrin is a known endocrine disrupting compound (EDC) found in biologically active concentrations in estuaries affected by urban runoff. Little is known about how elevated temperatures associated with climate change may affect the estrogenic activity of bifenthrin, particularly in species that exhibit temperature-dependent sex determination (TSD), Menidia beryllina. This study investigated the effects of temperature and bifenthrin exposure on reproductive output in M. beryllina across multiple generations. Fish in the parental generation were exposed to bifenthrin, ethinylestradiol (EE2 – positive control) and methanol (solvent control) at 22°C and 28°C for 14 days prior to spawning. Embryos in the F1 generation were exposed to EDCs as larvae (until 21 dph) and then reared to adulthood (8 months) in clean water at experimental temperatures. In all F1 treatments, elevated temperature resulted in fewer viable offspring. At the time of maturity, the F1 generation underwent spawning trials to assess reproductive output and offspring viability following larval exposure. Sex ratios of the F1 generation were influenced by elevated temperature and EDCs, resulting in alteration of adaptive TSD. Fish exposed to bifenthrin during development exhibited developmental deformities. Tissues were collected from each generation to assess the effects of these stressors on the expression of genes involved in reproduction and growth. Findings from this study will be useful in determining how EDCs will impact organisms and community structure in the face of global climate change.

647 Rainbow trout exposed to benzo[a]pyrene yields conserved microRNA binding sites in DNA methyltransferases across 500 million years of evolution

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Benzo[a]pyrene (B[a]P), a polycyclic aromatic hydrocarbon formed by the incomplete combustion of organic material found ubiquitously in the aquatic environment, is an established carcinogen and has been linked to multigenerational effects through alterations in DNA methylation patterns. The initial objective of this study was to examine the regulation of DNA methylation following acute (24 h) and prolonged (14 d) exposure to low (1 ng/L) and high (10 ng/L) B[a]P in rainbow trout. However, with the recent release of the rainbow trout genome, we were able to conduct a more detailed analysis regarding the regulation of the enzymes involved in DNA methylation; DNA methyltransferases (DNMTs). Specifically, we obtained the 3' untranslated region (3'UTR) of DNMT1 and DNMT3a, which are regulators of maintenance and de novo DNA methylation, respectively. Bioinformatic approaches were used to identify candidate microRNA (miRNA) that potentially bind to the DNMT1 and 3a 3'UTR, and would likely inhibit the transcript abundance. Following exposure, muscle and liver tissue was extracted and analyzed for global methylation, DNMT1 and 3a transcript abundance, DNA methyltransferase activity, and candidate miRNA abundance. Results indicated a significant decrease in the level of global methylation in both liver and muscle, with an associated decrease in total DNA methyltransferase activity and DNMT3a transcript abundance. Further, there was a significant increase in one specific candidate miRNA (miR29a) in both liver and muscle that was predicted to bind to DNMT3a. Taking a phylogenetic approach, the binding sites of miR29a to the DNMT3a 3'UTR was compared across numerous species, spanning fish to mammals, and results revealed a highly conserved binding motif which has been maintained since the vertebrate ancestor, approximately 500 million years ago. This research establishes that miRNA act as an essential mediator between the environment and DNA methylation patterns via DNMTs, which is further confirmed by a genomic regulatory mechanism that has been deeply conserved throughout evolution.

Fate and Effects of Metals: Regulatory and Risk Assessment Perspective

648 Relative Risk Approach: A methodology for the ecological risk classification of inorganic substances

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Under the Canadian Environmental Protection Act, 1999, Environment and Climate Change Canada (ECCC) and Health Canada assess and manage, where appropriate, risks of chemical substances to the environment and to human health. The Chemicals Management Plan (CMP) is a Government of Canada initiative that addresses approximately 4300 substances identified as priorities for assessment. In the next phase of the CMP (2016-2020), about 1550 substances remain to be addressed, including approximately 380 inorganic substances. Early activities to address inorganic substances include identifying assessment data needs, developing tailored strategies and approaches, and early stages of assessment drafting. In particular, a relative risk approach is under development for identifying and classifying the potential ecological risks of inorganic substances as lower or higher potential for ecological concern. Predicted environmental concentrations were derived through a modelling analysis and a monitoring data analysis. The modelling analysis considers the interpretation of key physical-chemical properties, the use of Domestic Substances List inventory update (DSL IU) volumes and National Pollutant Release Inventory (NPRI) data to model potential environmental releases. The monitoring data analysis considers surface water quality data from Canadian monitoring and surveillance programs. The relative ecological risk of individual inorganic substances and groups was determined through comparing the generated predicted environmental concentrations to selected predicted no-effects concentrations to determine an overall preliminary relative risk classification.

649 Northern Mines Need Marine Effluent Toxicity Tests for the First Time in Canada

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In 2002, the Metal Mining Effluent Regulations (MMER) came into force under the Canadian Fisheries Act. They applied to all mines (except diamond and coal) that release a freshwater effluent to either a marine or freshwater environment. Compliance testing for acute lethality utilized freshwater organisms in both cases as the effluent is regulated 'end-of-pipe' and not for reasons like determining effects of effluents mixed with the receiver or for predicting field effects. Currently, the fish Reference Method prescribed in the MMER is with rainbow trout (EPS 1/RM/13) and the invertebrate method prescribed is with Daphnia magna (EPS 1/RM/14). The invertebrate test is used now only for monitoring but will be elevated to a compliance test in the next regulation amendment. Until recently, there was no need to consider how to test the acute lethality of saline metal mining effluents in Canada. The development of Northern mining deposits with saline groundwater is creating a regulatory gap and now Environment and Climate Change Canada (ECCC) is developing compliance acute lethality tests for measuring saline effluent toxicity with marine species. Within a three year period, we are identifying appropriate test organisms, standardizing the test design, and validating the methods through an inter-laboratory study (or ring test). In choosing appropriate organisms, we have considered their range of salinity tolerance, their ability to be cultured under laboratory conditions, and their relevance to the Canadian environment, but most notably, the availability of existing methods. Work accomplished, and expertise developed, by other method standardization agencies will be leveraged to drastically reduce the time and cost necessary to develop standardized test methods. Working alongside the timeline for the MMER amendment, which will include these new test methods, Canadian toxicology laboratories will need training and accreditation for these tests.

650 Developing Copper and Zinc Standards for Ecosystem Health Protection in New Zealand's Freshwaters

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New Zealand is shifting from an effects-based approach to a standards-based appraoch for freshwater management. The New Zealand National Policy Statement for Freshwater Management (NPS-FM) has recently legislated nitrate and ammonia standards for all river environments – incorporating a risk-based approach and a national 'bottom line' which all waters are expected to achieve. These standards use a two-number criteria and management framework derived from on the Australian and New Zealand (ANZECC) risk-based methodology. The legislation requires the compulsory application of this framework for water classification. New Zealand's Ministry for the Environment is investigating incorporating metals (copper and zinc) in a future regulatory round, especially because increasing urbanisation and associated runoff is increasing stresses on local aquatic environments. For example, Auckland (New Zealand's largest city) is expected to increase it's population by 50% over the next 20 years but has limited space for expansion. Significant exceedances of ANZECC guidelines for copper and zinc in urban Auckland, and other urban areas, indicate that metals may be key contributors to poor ecological health in these urban areas. The proposed two-number approach for metals will take an additive toxic units (TU) approach to incorporate multiple metals present in urban streams – based on using suitable acute and chronic criteria and dissolved metals measurement. Monitoring data for urban areas showed that baseflow median dissolved Zn and Cu were 54% and 57% of total metals respectively, with dissolved chronic TUs of 2.5 and 8.1 for median and 90th percentiles, indicating that management practices would need to target dissolved fractions of Zn and Cu for baseline conditions. Limited event-related data is available to characterise acute exposure metals concentrations during rainfall-runoff and practical approaches will be required to monitor acute exposures to develop appropriate criteria, with passive samplers and other approaches being investigated. As the NPS-FM legislation requires management responses to reduce contaminant loads should the 'bottom line' be exceeded, suitable guidance will be needed to support toxicity identification, incorporation of toxicity modifiers (e.g., DOC, pH, hardness) and the implementation of source control and load reduction approaches.

651 Regulatory dexterity – The key to naturally fluctuating selenium concentrations

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Many watersheds in Colorado have naturally elevated selenium concentrations originating from geologic sources, but these elevated concentrations can be greatly influenced by other factors in the region. In a portion of the Cherry Creek watershed south of Denver measured selenium concentrations show strong seasonal variability and a reduction from upstream to downstream. Water samples collected throughout the watershed from 2013-2015 showed a reoccurring relationship with selenium concentrations decreasing as seasonal flows increased. In the winter, selenium concentrations exceed Colorado's acute water quality standards at the upstream sites. However, increased flows during the summer months – initially from spring runoff and runoff from lawn irrigation later in the summer – provide enough dilution to decrease selenium concentrations below the acute water quality standard. In addition, in months where the standards are being exceeded upstream standards at the downstream sites are attained as a result of selenium removal in a natural wetland. The fluctuation in selenium concentrations are of particular interest because there are no point source discharges contributing to selenium concentrations. Despite seasonally elevated selenium concentrations fish populations remain healthy; selenium related deformities were insignificant and reproduction appeared to be unaffected as multiple age classes (young-of-year, juveniles, and adults) for several species were observed throughout the study area. Data collected for this study were used to propose seasonally appropriate ambient-based site-specific standards during the Colorado basin hearing, which were approved by the Water Quality Control Commission. The seasonally appropriate site specific chronic standards range from 4.6 – 37.2 µg/L and acute standards range from 18.4 – 41.0 µg/L. There was less variability in fish tissue concentrations, with elevated levels even at sites with lower water column selenium. This has important implications with regard to implementation issues related to the release of the 2015 Draft Aquatic Life Ambient Water Quality Criteria for Selenium. Simply put, it is important to remember that no two systems are the same and while spatial variability is commonly observed, temporal variation must also be considered.

652 Understanding the behavior of Silver in surface and waste water systems: Evaluation of WHAM as a risk assessment tool

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Silver (Ag) is of particular interest due to its use in consumer products as an antimicrobial agent, leading to concerns over its unintended release into the environment. The release of Ag to natural environment through waste water treatment plants as well as direct inputs could result in increasing free metal ion concentrations causing toxicity. Understanding the behaviour of Ag, therefore, as is enters aquatic systems, is critical to evaluating its potential environmental risk. The aim of this research is to develop understanding of Ag interactions with strong ligands such as dissolved organic matter (DOM) and sulphide compounds present in water (fresh and wastewater). A wide range of DOM sources (ranging from reference material to sewage effluents) as a well as raw sewage were used to understand Ag complexation with DOM at different pHs (4-10). Ag ion-selective electrode was used for potentiometric titrations to determine Ag binding to DOM and the measured Ag^+ was then compared to WHAM predictions. In addition, DOM was characterized optically and sulphide (inorganic & organic) analysis was also conducted on the samples. WHAM predictions of the Ag binding to DOM sources agree well with experimental results at pH 8, with the exception of raw sewage, which exhibited much stronger binding. This very strong binding could be partly, but not completely, explained by binding to thiol– and amino–containing ligands. A conditional binding model, comprising five ligands and using a structured formulation of binding strengths and site densities, was successfully fitted to the raw sewage titration data. The model fitting suggested that the strongest Ag–binding sites were comparable in binding strength to thiol groups, but that the sample contained relatively weak ligands that nevertheless appeared to be stronger binders of Ag than either amino or carboxylate groups. This suggests that multi-dentate binding of Ag may be important in determining the overall binding strength in raw sewage. At pHs below 8, WHAM under predicts Ag binding for some samples, which suggests that incorporation of additional strong ligands could improve WHAM predictions of Ag speciation in the environment. This will allow for development of more accurate and robust predictive tools for Ag risk assessment and regulation in aquatic systems.

653 Comparison of Aluminum Aquatic Life Criteria Approaches

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The effects of water chemistry on the aquatic toxicity of metals have been an area of research since the Environmental Protection Agency's (EPA) water quality criteria (WQC) were first developed. A partnership among industry, academia, and government utilized research advancements in the aquatic toxicity of metals to effectively develop EPA' national water quality criterion for freshwater copper to protect aquatic organisms. This effort used the Biotic Ligand Model (BLM) to address the bioavailability of metals in aquatic systems and their acute toxicity to fish. The BLM adjusts the water concentration that causes acute toxicity of metals to aquatic organisms by calculating the relative binding affinity of cations in the water to the biotic ligand (eg., gills or any other active site) based on the geochemical conditions of the site versus a single value for the criteria. Similarly, in developing updated aluminum criteria, EPA is comparing several approaches that reflect water quality condition impacts on toxicity including: (1) existing complete 10 parameter BLM(s), (2) a simplified BLM approach where pH, hardness, dissolved organic carbon, temperature values were entered and other parameters use default values, and (3) multi-linear regression equations using pH, hardness, and dissolved organic carbon parameters. Comparisons were conducted to facilitate evaluation of the various approaches for criteria development.

654 Is total recoverable aluminum relevant for water quality criteria?

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The national recommended ambient aquatic life water quality criteria (WQC) for aluminum in the United States are dated and the aluminum form that they are expressed as is potentially not relevant from a toxicological perspective. The chronic criterion of 87 mg/L is based upon total recoverable aluminum and is applicable for waters with pH between 6.5 and 9.0. Numerous studies have demonstrated that the toxicity of aluminum to aquatic organisms is affected by the chemistry of the exposure water, with pH, dissolved organic carbon (DOC), and water hardness being important toxicity modifying factors. Water chemistry also influences aluminum solubility, and this can be especially relevant because toxicity studies have demonstrated that freshly precipitated aluminum hydroxides can contribute to toxicity. Therefore, toxicity cannot be explained on the basis of dissolved aluminum alone. The conundrum is that toxicity cannot necessarily be explained on the basis of total recoverable aluminum, either. In laboratory toxicity tests, total recoverable aluminum represents dissolved aluminum and potentially some forms of mineral precipitates. In natural settings, surface waters are likely to contain suspended solids, and because aluminum represents approximately 8 percent of the Earth's crust it is expected that solids in the water column (e.g., silt and clay) will contribute to total recoverable aluminum measurements. While the methodology for determination of total recoverable aluminum will not necessarily solubilize all solids, it is likely that solids will contribute to the total recoverable aluminum concentration. To evaluate situations where suspended solids, which are not likely to be bioavailable, may contribute to total recoverable aluminum measurements, monitoring data were compiled from the NWIS (USGS) database. The type of data compiled consisted of dissolved and total aluminum, pH, DOC, major ions, total suspended solids, and suspended sediment concentrations. These data were used to evaluate cases where aluminum exceeds mineral solubility and to identify situations where total recoverable aluminum measurements are likely to include contributions from suspended solids. Results of this analysis suggest that WQC based upon total recoverable aluminum are overly conservative because of the contribution from suspended solids in the water column. An alternative, toxicologically relevant approach to aluminum WQC is warranted.

655 Field study of coarse sample pre-filtration as a compliance tool to minimize false-positive criteria exceedances for aluminum

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Compliance with water quality standards for metals is typically evaluated using either dissolved or total recoverable metal concentrations. For most metals, dissolved fractions best represent biologically available fractions, and so dissolved measurements are typically preferred. Aluminum regulatory criteria generally are based on the total recoverable metal because some insoluble colloidal phases can contribute to toxicity. However, the heat and acid digestion used in a total recoverable assay also measures metals associated with non-toxic particulate mineral phases, and so will likely overestimate concentrations of metal that would be biologically available and potentially toxic to aquatic life. This overestimation may result in "false positive" exceedances of aluminum criteria when samples contain elevated suspended solids comprised of non-toxic particulate aluminum. To help address this issue, aluminum criteria in New Mexico include a coarse sample pre-filtration step, with a recommended filter size of 10 µm prior to measuring total recoverable metal to minimize the amount of bias potentially incorporated by measurement of non-toxic mineral phase metal. This study evaluated the effects of different pre-filter pore sizes on aluminum concentrations and to evaluate the potential for pre-filtration to affect any potential toxicity of the water samples collected from stormwater retention ponds in Colfax County, New Mexico. The acute toxicity of the unfiltered water was evaluated for all sites and no toxicity was detected at concentrations of total recoverable aluminum as high as 55.8 mg/L, which is well above the acute water quality criteria based on the hardness of each sample. However, pre-filtration using a 1 µm filter reduced the aluminum concentrations to below criteria concentrations. The coarser aluminum particulates retained by this pre-filtration step are likely to be predominantly mineral (i.e., from native soils) in nature and not present in a bioavailable form. In addition, the molar aluminum-to-silica ratios showed that mineral phase aluminum was still passing through the 5 and 10 µm filters while the 1 µm filter likely removed these particulate aluminum phases. We conclude that pre-filter pore sizes as small as 1 µm are an acceptable alternative for removing larger non-toxic aluminum particles that would otherwise represent a false-positive comparison against regulatory criteria.

Engineering, Toxicology and Risk Assessment Guidance for Sediment Evaluation at Dam Removal Sites

656 Dam removal analysis guidelines for sediment

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American Rivers reported in 2014 that 1,185 dams have been removed in the United States since 1912, and that the majority of the dams (971) were removed within the past 20 years. Dam removal may be a preferred alternative for sites where the original project benefits are no longer needed or can be replaced with a reasonable alternative. Examples include sites with aging or abandoned infrastructure with hazard issues or intakes no longer operational due to sedimentation. Removal can often accomplish increased environmental benefits that can in part be obtained by reconnecting the supply of sediment, wood, and nutrients to areas from the upstream watershed to the river downstream of the dam. Because dams are built on both small and large rivers with a wide range of sediment characteristics, dam removal can result in varying levels of impacts. While some sites have negligible sediment that does not warrant robust analysis, other sites may have potential contaminants or required staged removal to control the rate of sediment release. Stakeholders, regulating agencies, and technical staff may not agree on what constitutes significant sediment impacts, and what level of information is needed to make decisions regarding sediment management. Existing manuals do not provide a framework or guideline for determining the level of analysis needed, the significance of sedimentation issues, or certainty that can be attained with available analysis tools. The U.S. Subcommittee on Sedimentation has recognized the need for a technical guideline to address sediment analysis for dam removal investigations. The new guideline utilizes a risk-based concept to determine the level of sediment data collection, analysis, modeling, and management necessary to evaluate and plan dam removal projects. The risk is defined as the product of the probability (e.g. likelihood) of a sediment impact and the consequence of the impact should it occur. The probability is generated by physical data at the site, whereas the consequence list is developed through collaborative discussions with project partners and stakeholders. The greater the risk, the greater the recommended level of sediment data collection, analysis, modeling, and management. Based on the risk determination, the guideline provides users a pathway to identify types of analysis and monitoring strategies for a given dam removal project.

657 Analyses of fine sediment transport for a large dam removal project: an empirical approach

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Potential accumulation of pollutants in reservoirs is normally associated with fine sediment deposits. The first step to understand the potential impacts of the downstream release of these sediments following dam removal is to understand the physical and chemical composition, volume, and spatial distribution of the sediment deposit, and from there, to forecast the sediment transport dynamics if they are released downstream. Unfortunately there are currently no reliable numerical models to accurately simulate the dynamics of the release of these fine sediments, mostly because their release following dam removal is often driven by rapid erosional process not addressed by traditional sediment transport theory. However, precise quantification of fine sediment transport is rarely necessary to evaluate potential environmental impacts of alternative scenarios. Using the removal of Matilija Dam in southern California, USA as an example, we quantify the likely magnitude of suspended sediment concentration and the duration of associated downstream impacts, two important parameters in assessing alternatives. Using a two-phase conceptual model established based on our understanding of the physical process under such conditions, the analyses first estimate the general magnitude of suspended sediment concentration and duration of impacts based on field and experimental data; they then quantify the duration of impacts under both worst-case and reasonable assumptions according to the underlying physics. For rapid sediment release scenarios, initial suspended sediment concentrations are likely to approach one million mg/L, persisting for a few hours to no more than a couple of days. Suspended sediment concentrations are expected to decline approximately exponentially after the initial peak, reaching background levels in this naturally high-sediment yield watershed within a few hours to a few days, provided that sufficient flow is available. The general method used in the analyses should be useful for similar dam removal projects, allowing the stakeholders to conduct further impact analyses and/or choose amongst dam-removal alternatives for implementation.

658 Unleashing History: Understanding Dynamic Processes Affecting Sediment Toxicity During Dam Removal

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Assessment of sediment during dam removal requires a strong understanding of the dynamic processes that are often overlooked as part of a traditional sediment risk assessment. Historical deposition of sediment in reservoirs results in accumulation of a broad range of sediment types depending on hydraulic events (e.g., floods, overland flow, erosion) and contaminants based on regional activities. In addition the type of sediment deposited may contain a wide range of organic materials and the biogeochemical processes that can be affected by regional geology. This presentation will emphasize the need to consider complex dynamic processes that are perturbed during dam removal and mobilization of sediment. These processes include changes in redox status, microbial activity that can affect nutrient status and contaminant bioavailability. Laboratory studies can be conducted to provide information that can be used to assess risk of sediments during dam removal, however the studies must consider temporal trends so that data can be applied in a predictive manner. For example, speciation of metals in the Tennessee Valley Authority Fly Ash spill was critical for understanding risk to benthic invertebrates and to predict release of Se and As ions. Within laboratory studies the temporal trends related to redox potential and metal speciation must be evaluated to predict bioavailability of metals in the field. Capturing this information requires the use of methods including porewater passive samplers coupled to bioassay methods that generate kinetic information on uptake and chronic effects. Case studies discussed will include processes observed during release of sediment during accidental breaches of dams as well as dredging of sediments.

659 Best Practices to Inform Risk-based Decisions for the Management of Dam Impounded Sediments

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In recent years an increasing number of Dams have been identified for removal either as a consequence of concerns over safety, diminished economic viability, and/or a desire to improve river function, etc. Sediments trapped behind dams slated for removal must be evaluated to establish appropriate management alternatives. Typically sediments impounded behind dams are organically enriched silts and clays with a propensity to bind contaminants and are generally reflective of the history of upstream land use activities, including legacy contaminants associated with agricultural and or industrial practices. Establishing viable management alternatives for these sediments prior to dam removal requires an evaluation of the potential ecological and human health risk posed by the proposed management action. As with any risk based evaluation, development of a site conceptual model is a critical first step to identify potential receptors of concern and the nature and magnitude of potential exposure pathways. Once these elements are understood, appropriate conservative screening tools maybe applied to identify potential risks that might require further evaluation in a more detailed site-specific assessment. This presentation provides an overview of a tiered, risk-based approach to include considerations for development of a site conceptual model, collection of data for initial screening, application of conservative screening tools, as well as application of a more detailed site specific assessment (sediment toxicity and bioaccumulation studies) should it be required.

660 What are the potential contaminants in sediment trapped behind low head dams in the Midwest?

J. Frey, USGS

There are 1000's of low head dams in the Midwest that no longer are used for their original purpose, typically power supply for grist mills, slackwater canal systems, or water supply. These low head dams create recreational safety problems with multiple people killed or badly hurt each year. Additionally, low-head dams have several ecological impacts, including degraded biological communities, obstructed fish passage, and accumulation of contaminated sediments. There are calls for low head dam removals based on these safety and ecological impacts but, there are few published studies that assess the contaminants that could be found in the sediments trapped behind these dams. The USGS Midwest Stream Quality Assessment collected data from July and August 2013 on contaminant concentrations in water and sediment at 27 sites on small and wadable streams and rivers. Sediment samples were analyzed for a suite of wastewater-associated compounds, hormones, and halogenated compounds now or previously in commerce (e.g., chlorinated pesticides, brominated flame retardants, PCBs). The most frequently detected compounds in the 27 samples included indole and 3-methyl-1H-indole (100% detection with a maximum concentration of 1,960 and 236 ug/kg, respectively), dieldrin (85.1% detection with a maximum concentration of 2.26 ug/kg), beta-sitosterol (96.3% detection and a maximum concentration of 48,900 ug/kg), p-cresol (92.6% detection and a maximum concentration of 14,400 ug/kg), 2,6-dimethylnaphthalene (92.6% detection and a maximum concentration of 126 ug/kg), dieldrin (85.2% detection and a maximum concentration of 2.26 ug/kg), and beta-stigmastanol (81.5% detection and a maximum concentration of 4,080 ug/kg). Although these samples were not collected behind dams, these data indicate potential contaminants of sediment behind low head dams that could be displaced if the dams were removed. As such, the results help focus what if any additional work may be important at individual dam removal sites.

661 Screening-level evaluation of potential toxicity risks from release of sediments behind four dams on the Klamath River, Oregon and California

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One of the most common management concerns related to dam decommissioning is the release, transport, and ultimate fate of sediments stored behind the dams. Contaminants in reservoir sediment are a particular challenge because they are expensive to collect and analyze and therefore are often understudied; their effects on humans and biota are not well understood especially in mixtures; and societal pressures may amplify the perception of their risk and the importance of decision making regarding their ultimate disposition. This study describes a screening-level evaluation to assess possible risks from contaminants in sediment behind four hydroelectric dams on the Klamath River in southern Oregon and northern California that are proposed for removal. The study followed the tenets of the interagency Sediment Evaluation Framework and the Dredged Material Management Program that guide decision making for dredging and disposal of sediments in the Pacific Northwest. The study generated multiple lines of evidence that were compared to five relevant exposure pathways of biota and human receptors to identify potential adverse effects. Lines of evidence included analysis of existing data that informed subsequent sample design; an inclusive suite of chemical analyses from multiple cores from each reservoir and downstream receiving waters; comparison of chemical data with established benchmarks; laboratory bioassays of aquatic biota to sediment and elutriates; bioaccumulation studies, and analysis of tissues in resident fish from the reservoirs. Short term (< 1 yr) exposure pathways included humans and aquatic biota via contaminants flushed downstream. Long term (> 1 yr) exposure pathways included humans and biota via deposits in reservoir terraces and river banks, in river beds, and in marine near shore areas; and humans and biota under current conditions via consumption of resident fish from the reservoirs. Results demonstrated that, while a variety of contaminants were detected at low levels, there were no chemicals present at levels with potential for significant adverse effects or that would preclude their release into downstream or marine environments. In this case, employing multiple lines of evidence for chemical toxicity and multiple exposure pathways provided evidence that significant adverse effects would be avoided if the Klamath Dams are decommissioned and the sediments are allowed to flush downstream.

662 Milltown Dam Removal Case Study

D. Booth, Booth Consulting

Milltown Dam, located on the Clark Fork River upstream of Missoula, Montana, was originally constructed in 1907 to provide hydropower-generated electricity for a sawmill. During the past century, metals-impacted sediment washed down from historic mining and smelting operations in the Butte/Anaconda area filling Milltown Dam's reservoir and resulting in arsenic contamination of the local groundwater. In 2004, as part of remediation of the larger Clark Fork River Basin Superfund Site, the U.S. Environmental Protection Agency ordered the removal of Milltown Dam and approximately 3 million cubic yards of reservoir sediment. Design and implementation of the Milltown dam removal/reservoir restoration program required orchestration of many factors involving State, tribal and federal agencies, as well as numerous community stakeholders. Of particular importance was sediment management decision-making which required the integrated effort of specialists in hydrology, engineering, environmental assessment and management, toxicology, and risk assessment. The project, which was completed in 2011, represents one of the most complex and intensively studied dam removal efforts completed to date and therefore provides lessons learned and best practices for use in future projects and in development of Dam Removal Analysis Guidelines for Sediment.

663 Sediment Management: The Intersection of Policy and Practice at Dam Removal Sites

S. McClain, American Rivers

Dam removal is one of the fastest, most effective ways to restore a river. However, for natural resource managers, watershed organizations and others, determining how to remove a dam and navigate things like the regulatory process can seem daunting. One of the more challenging issues that can define a project's level of complexity is the manner in which sediment must be managed. Sediment is one of nature's building blocks, moving and shaping the earth as it weathers and erodes rocks. Yet, as it becomes trapped behind dams, we struggle with the question of how to re-establish the beautiful, chaotic function of rivers in a way that avoids unacceptable degradation. The answer is not often as simple as letting the data dictate a path forward. Federal and state regulatory processes can have significant influence over sediment management options through the varied application of regulations tied to Clean Water Act 404 permitting and 401 water quality certifications. Though several states (e.g., New Hampshire and Massachusetts) have developed guidance for managing sediment at dam removal sites, many still view these projects through the same regulatory lens as traditional development projects. New guidance from the EPA on Water Quality Standards and the Bureau of Reclamation and U.S. Subcommittee on Sedimentation guidelines are at the forefront of efforts to address these inconsistencies. During this talk, we will explore how the intersection of sediment science and policy have influenced the practice and how rollout of these guidelines will contribute to and influence the management of sediment at future dam removal sites.

Integrating Chemistry and Biology in a Landscape Context to Reveal Potential Causes of Endocrine Disruption

664 Endocrine disrupting compounds in the Chesapeake Bay Watershed – Where are we going? Where should we go?

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USGS Scientists form across teh countray are tasked with establishing a national frameowrk to evaluate endocrine disrupting compounds and their effect on fish. This framework was applied directly to ongoing efforts as part of the USGS Chesapeake Bay research on fish health. In addition to endocrine disruption, Fish health issues include increased incidence of infectious disease and parasite infestations, feminization of male fish, reduced reproductive success and recruitment, and liver and skin tumors. Identifying and quantifying the sources, fate, transport, and distribution of EDCs throughout the Chesapeake Bay Watershed are a necessary first step in assessing exposure to fish. Chemical data are being collected and compiled from strategically selected stream sites in the Chesapeake Bay Watershed to: 1) address the multiple factors affecting fish health by focusing on the complex interactions of pathogens/parasites, land use and chemical exposure and 2) define sources, pathways and timing of chemical exposure to various life stages of fish for chemicals identified as causing potential adverse effects on fish health. Water and bed sediment are being collected and analyzed for over 200 potential EDCs (including hormones, pesticides, personal care products, and legacy pollutants) and bioassays are being assessed for biological activity such as estrogenicity. Initial sampling is focused on routine data collection from six sites in the Chesapeake Bay watershed representing a wide range of agricultural land use conditions, during both baseflow and stormflow conditions. Future sampling will include assessments of urban areas. Chemical and bioassay sampling are coordinated with companion biologic assessments (including histopathology, plasma vitellogeninin, intersex and other measurements) in order to assess exposure of fish to chemical contaminants at different times during the year and during different flow conditions. In addition, chemical data are being compiled from existing sources (including previous studies of EDCs and pesticides) in order to expand the geographic and temporal coverage of chemical and biologic analysis. Results from this study will be used to help understand sources and exposure pathways of EDCs to fish, and to develop management strategies to improve fish health in the Chesapeake Bay watershed.

665 Contaminants in the Chesapeake Bay watershed: A synthesis of data from a decade of studies using passive samplers

D.A. Alvarez, USGS-CERC / Physiology and Biochemistry; V.S. Blazer, USGS

For more than a decade, adverse effects associated with exposure to endocrine disrupting chemicals (EDC) have been observed in the Chesapeake Bay watershed. These effects have included major fish kills in the Potomac River drainage and increased rates of intersex in male smallmouth bass. In response to these biological issues, studies were conducted using passive sampling devices to identify organic contaminants in the water that may be responsible for the biological effects. These findings have been reported as parts of numerous smaller independent studies with no linkage between studies. In an effort to gain a better understanding of the overall chemical profile in the watershed, a synthesis of chemical occurrence data collected at sites co-located with fish health studies has been conducted. The majority of the study sites have land-use characteristics ranging from largely agricultural to mixed agricultural/small urban centers. Due to the prevalence of agriculture, the herbicides atrazine and metolachlor were two of the most frequently detected chemicals in the watershed. Estrone, likely from livestock and manure application as fertilizer, was commonly detected. Polycyclic aromatic hydrocarbons (PAHs) had a ubiquitous presence and the legacy organochlorine pesticides were frequently detected; however, generally at low nanogram per liter concentrations.

666 A landscape-based approach to assess the effects of exposure to complex chemical mixtures in the Shenandoah River watershed

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Aquatic habitats are often contaminated with complex mixtures of legacy and emerging chemicals. Characterizing the biological effects of exposure to these mixtures is an ongoing challenge. Identifying landscape-specific contaminant profiles and measuring the effects of exposure to these mixtures may reveal patterns useful for chemical regulation and management of ecosystems. Here, we employed an integrated chemical and biological analysis to measure differences in chemical mixtures and associated biological effects in watersheds with different landuse patterning. Adult male fathead minnows (Pimephales promelas) were exposed to water from four different locations within the Shenandoah River watershed (VA, USA) using flow-through mobile laboratories. The exposure locations were chosen to capture unique landuse in surrounding watersheds, including agricultural, municipal, mixed-use, and forested sites. Endpoints from multiple levels of biological organization were measured following exposure, including condition factor, GSI, number of nuptial tubercles, plasma vitellogenin concentration, and hepatic genome-wide expression profiles. Water samples were taken every 7 days during the fish exposure and analyzed for 460 chemical constituents. Each location had a unique chemical profile that was generally consistent with landuse in the surrounding watershed. Whole-organism and molecular responses also differed between the locations. At the agricultural site, survivorship was significantly reduced. Fish exposed at both agricultural and WWTP impacted sites had a reduced number of nuptial tubercles and decreased GSI, indicative of reproductive endocrine disruption. However, transcript biomarkers of estrogen exposure, including er1, er2, ar, vtg1, and vtg3, showed little to no differential expression in exposed fish, suggesting these organisms did not experience estrogenic endocrine disruption. Transcriptomics followed by pathway analysis revealed that processes related to immune function, cholesterol synthesis, and metabolism were affected in fish exposed at agricultural and WWTP impacted sites. These data provide insightful hypotheses regarding the specific effects of exposure to different types of complex mixtures and demonstrate the value of our complex mixture/landscape research approach.

667 The reality of combining chemical analyses and common bioassays for effects-directed analysis of endocrine disrupting chemicals in environmental water

J.C. Brennan, USGS / Columbia Environmental Research Center; D.A. Alvarez, USGS-CERC / Physiology and Biochemistry; R.W. Gale, J.K. Leet, D.E. Tillitt, USGS / Columbia Environmental Research Center

Endocrine disrupting chemicals (EDCs) are known to negatively impact reproductive, immune, and other endocrine-related processes in fish and other animals. Unexpected die-offs, skin lesions, and intersex in fish may be due to exposure to EDCs. Effects-directed analysis (EDA) is a technique utilized to discover the identity of biologically-active chemicals such as EDCs from complex mixtures including waters. In addition to discerning if the endocrine disruption in a particular environment is plausibly chemically-induced (i.e. male fish displaying intersex in a watershed), EDA can guide the identification of the responsible EDCs and also help rule out candidates not responsible for the observed biological activity or effect(s). However, EDA can be a labor-intensive process with a high likelihood of bioactive compound loss. EDA also is sometimes not possible due to sample volume limitations or low concentrations of EDCs often found in environmental waters. We assessed the ability of several bioassays (the well-known transactivation chemically-activated luciferase expression (CALUX) and the bioluminescent yeast estrogen/androgen screening (BLYES/BLYAS) cell bioassays, and the relatively new transgenic (CYP19a1b)-GFP zebrafish bioassay) to guide isolation of different types of EDCs in a conceptual EDA of natural and wastewaters. An EDA framework (known as the “decision tree”) was developed and a literature review of EDC concentrations in U.S. freshwaters was conducted as a basis for the bioassays’ detection capabilities. We found that, when compared to the BLYES and zebrafish screening assays, the CALUX estrogen cell assay had the greatest rate of detection above half-maximal activity for the majority of estrogens in natural waters post-EDA but all three assays had high detection rates in wastewater sources. The CALUX cell androgen bioassay had the greatest rate of detection for the majority of androgens in both natural and waste waters. There was no difference between the CALUX and yeast cell assays for detection of progesterone whether in natural or waste waters with high detection rate for both assays. The majority of EDCs had a higher rate of detection in all bioassays in wastewater vs natural waters, but for one EDC (genistein), none of the bioassays were suitable for EDA. The CALUX cell bioassays, in particular, when utilized for EDA allow detection of certain EDCs in water sources, while detection of other chemicals offers more challenges.

668 Severe intersex in wild fish is reduced to background levels after municipal wastewater treatment plant upgrades

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Impacts on fish exposed to effluent from municipal wastewater treatment plants (WWTP) are a concern globally, with endocrine disruption being one of the most prominent effects observed. The objective of this study was to assess whether major treatment upgrades at a WWTP would reduce endocrine disruption previously observed in wild fish. The WWTP located in Kitchener, Ontario, is a conventional activated sludge plant that discharges effluent into a heavily urbanized reach of the Grand River. Multiple years of data have been collected for a sentinel fish species, the rainbow darter (Etheostoma caeruleum), associated with the Kitchener WWTP outfall since 2007. Intersex in male fish has been the most consistent effect observed, and this has been associated with reduced reproductive success. In 2012, major upgrades took place to convert the WWTP from non-nitrifying to a fully nitrifying activated sludge treatment plant. Effluent quality improved with declines in contaminants such nutrients and select pharmaceuticals, which are also reflected in the river water downstream the WWTP. The effluent estrogenicity was measured as high as 17 ng/L total 17β-estradiol equivalents prior to treatment changes but has decreased with implementation of the upgrades. This improved effluent quality was also reflected in the food web with $\delta^{15}N$ values measured in fish tissue which are now similar to fish from upstream sites. Intersex was assessed in male rainbow darter downstream the Kitchener WWTP in three consecutive years post-upgrade. There was a reduction in intersex incidence by as much as 85%, suggesting that the rainbow darter is responding to process upgrades. This is one of few studies to document a recovery of a wild fish population after improved effluent quality from a municipal WWTP.

669 Causal Analysis for Gonadal Development in Wild Redeye Mullet (Liza hematocheila)

J. Hu, Peking Univ

Reproductive abnormalities (gonadal intersex) have been observed in wild fishes and attributed to endocrine disrupting chemicals, but the specific causes are still unclear. To unravel the mystery, a forensic analysis utilizing field and laboratory studies is necessary to determine the causative agent(s). Investigations of potential intersex incidences and reproductive effects by pollutants at environmentally relevant concentrations is challenging due to the fact that 1) large sample sizes are required to achieve results with statistical power at environmental concentration, and 2) conventional histopathological observations of intersex can be easily overlooked due to the limited observation area of the testes and time required in this process. Therefore, at first we developed pMOSP1-EGFP transgenic Japanese medaka, which is a specific and sensitive biosensor for indicating intersex occurrence in male medaka fish by green fluorescence protein (GFP). Then we used this transgenic Japanese medaka for screening the chemicals which can induce intersex at environmental relevant chemicals, and find a potential chemical which could be a causal agent to induce the intersex of wild fish from the Liaodong Bay. Our approach provided a methodology to well understand the adverse outcomes of ecological health, and emphasized the urgent need to control the causal agent to avert ecological consequences of wild fishes.

671 Equol Induces Gonadal Intersex in Japanese Medaka (Oryzias latipes) at Environmentally Relevant Concentrations: Comparison with 17β-Estradiol

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Equol is present in the aquatic environment via livestock waste and runoff discharge; however, it remains unclear whether it can induce gonadal intersex in fish at environmentally relevant concentrations. This study evaluated adverse effects of equol on gonadal development by exposing transgenic Japanese medaka (Oryzias latipes) from hatching for 100 days. Equol induced intersex incidence in male medaka in a dose-dependent manner, and the benchmark dose corresponding to 10% intersex incidence (BMD_{10}) was 11.5 ng/L (95% confidence interval (CI): 5.8 ng/L, 19.8 ng/L), which was comparable to the required dose of 17β-estradiol (E2β) (9.0 ng/L, 95% CI: 6.6 ng/L, 11.0 ng/L). Since the BMD_{10} of equol was analogous to concentrations found in the aquatic environment, equol contamination would likely induce intersex in wild fish. Equol exposure resulted in reduced plasma 11-ketotestosterone (11-KT) concentrations in male medaka at 1.3 ng/L, while reduced plasma 11-KT concentrations were observed at a relatively high concentration (6.4 ng/L) of E2β. Such anti-androgenic property could partly explain the comparable potency of equol with that of E2β to induce intersex at relatively low concentrations, although the binding affinity of equol to medaka estrogen receptor α (EC_{50} 939.4 nM) was 230-fold lower than that (4.07 nM) of E2β. Significantly lower fertilization (p = 0.016) and hatchability rates (p = 0.013) were observed in the moderate to severe intersex males exposed to equol. This study for the first time demonstrated that equol could induce intersex in medaka fish at environmentally relevant concentrations.

Use of Modeling Tools to Determine Environmental Concentrations from Environmental Passive Samplers

672 Multiple coating thicknesses for sampling of organic pollutants – linear regressions confirm equilibrium even in challenging environmental media

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Partitioning-based sampling methods are increasingly used within environmental analytical chemistry and environmental monitoring. Such methods can be designed and operated in many different ways and can then yield a large variety of measurement parameters including time integrative concentration, freely dissolved concentration, chemical activity and equilibrium partitioning concentration in lipid. The analyte transfer from the environmental matrix into the polymer has several analytical advantages, but also leads to the crucial question on how to convert the measured mass accumulated in the sampler to a meaningful environmental parameter? This presentation focuses on equilibrium sampling, which allows for simple conversions using partition coefficients – even when sampling very complicated matrices. Parallel sampling with coatings of the same polymer but varying coating thicknesses can be used to conduct and confirm valid equilibrium sampling. Proportionality between analyte and polymer masses will then indicate (1) equilibrium partitioning, (2) the absence of substantial surface artefacts such as adsorption rather than absorption or abrasion and (3) the absence of depletion. It can thus provide QA/QC, including validity of sampling assumptions, without using performance reference compounds. Equilibrium concentrations of the analytes in the polymer are determined as the slopes of the proportionality lines, which integrates all measurements and also provides error estimates. The working principle of this approach will be presented together with a number of examples that include (i) several sampler formats, (ii) challenging environmental media such as sediment, soil, tissue, sludge and air, (iii) ex situ and in situ equilibrium sampling and (iv) targeted instrumental analysis of legacy pollutants and non-targeted screening of complex environmental mixtures. The challenge of reaching equilibrium within a reasonable time span will be addressed for different compounds and media, together with the analytical performance of equilibrium sampling in terms of sensitivity, precision and accuracy.

673 Actively shaken in situ passive sampler for measuring pore water concentrations of hydrophobic organic compounds

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Passive sampling for the measurement of freely dissolved concentrations of organic pollutants in sediment pore water has emerged as a very promising approach, but in situ measurements are complicated by slow mass transfer of strongly hydrophobic compounds. In this research, we addressed the challenge of slow mass transfer by disrupting the external aqueous boundary layer around an in-situ passive sampler using periodic mechanical vibration. Laboratory experiments were performed using PAH and PCB impacted field sediments in order to compare the approach to equilibrium in PE passive samplers under static, well-mixed, and differently shaken passive sampling modes. Low-cost vibrating motors were used to provide the mechanical vibration of passive samplers. Passive samplers were enclosed in stainless steel mesh and were attached to motors like radial fins. We also performed numerical modeling of the mass transfer process to mechanistically explain our observations and optimize the duration and periodicity of the vibration to minimize the need of power to drive the vibration motors. We demonstrate through laboratory experiments and numerical modeling that periodic shaking in-situ greatly enhances overall mass transfer and reduces the difference in the extent of equilibrium achieved between a well stirred laboratory equilibrium and in-situ periodically shaken passive sampler. Vibration also reduced the errors involved in corrections for non-equilibrium based on fractional loss of performance reference compounds. Thus, greatly improving the accuracy of pore water measurement of strongly hydrophobic compounds. The proposed platform presentation will present the experimental results and numerical modeling of PAH and PCB uptake by PE passive samplers under static, well-mixed, and periodically shaken passive sampling modes.

674 Evaluating the precision of passive sampling methods using PRCs in the water column

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Low-Density polyethylene (LDPE) sheets are often used as passive samplers for aquatic environmental monitoring to measure the dissolved concentrations of hydrophobic organic contaminants (HOCs). HOCs that are freely dissolved in water (C_{free}) will partition into the LDPE until a thermodynamic equilibrium is achieved; that is, the HOC's chemical potential in the passive sampler is the same as its potential in the surrounding environment. Unfortunately, achieving equilibrium for high molecular weight or highly hydrophobic compounds can take several months or even years. One way to evaluate equilibrium status or estimate the uptake kinetics is by using performance reference compounds (PRCs). PRCs are partitioned into the LDPE prior to deployment and based on the fraction of each PRC lost during deployment, a sampling rate (R_s) or a fractional equilibrium (f_{eq}) can be determined for the target HOCs. Assuming equilibrium or applying a model using PRC data to estimate C_{free} concentrations may affect the precision of the measurement. In this work, we investigate how using different PRC models in different states of equilibrium affects the measured C_{free} concentrations and their precision for a suite of PCBs. To assess these models, four different thicknesses of LDPE passive samplers were co-deployed for 28 days in the water column at three sites in New Bedford Harbor, MA, USA. Following the deployments, the percent of PRC lost ranged from 0-100%. Fractional equilibrium decreased with increasing PRC molecular weight as well as sampler thickness. These data allow C_{free} comparisons to be made in two ways: (1) comparing C_{free} derived from one thickness using different models and (2) comparing C_{free} derived from the same model using different thicknesses of LDPE. Overall, a total of 27 PCBs (log K_{OW} ranging from 5.07 – 8.09) were measured at C_{free} concentrations varying from 0.05 pg/L (PCB 206) to about 200 ng/L (PCB 28) on a single LDPE sampler. Relative standard deviations (RSDs) for total PCB measurements using the same thickness and varying model types range from 0.04-12% and increased with sampler thickness. Total PCB RSD for measurements using the same model and varying thickness ranged from: 6 – 30%. No RSD trends between models were observed but RSD did increase as C_{free} decreased. These findings indicate that existing models yield precise and reproducible results when using LDPE and PRCs to measure C_{free}.

675 Why kinetics matter: the use of a numerical diffusion model for determining fractional equilibrium in passive samplers at varying flow conditions

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Passive samplers have been effectively used to measure the freely-dissolved concentrations of hydrophobic organic contaminants in natural waters. Because rates of chemical uptake vary due to molecular size and ambient water conditions, including water boundary layer (WBL) thickness and the ratio of sampler size-to-water volume, the time to equilibrium is both compound- and water condition-specific. The use of pre-added performance reference compounds (PRCs) can be used to correct for this disequilibrium allowing for more accurate estimates of in situ contaminant concentrations. A physically based, one-dimensional diffusion model can be used to estimate water boundary layer thickness and correct for disequilibrium for multiple chemicals. In situ field measurements typically allow for limited concentration measurements (beginning and end of sampler deployment). In this study, synchronous fluorescence was used in polyethylene passive sampler laboratory experiments to measure multiple (~30) absorption and desorption concentrations of phenanthrene over time in order to verify the isotropic nature of the diffusion process in polyethylene (PE). A numerical diffusion model was created and found to be in good agreement with the absorbing and desorbing phenanthrene concentrations. In subsequent laboratory experiments, PE was spun at varying speeds (0, 1, 30, 80 cm/s) in order to mimic a range of environmentally-relevant WBL thicknesses and the desorption rates for pre-added pyrene and absorption rates for phenanthrene and benzo(a) pyrene were measured using synchronous fluorescence. The pre-added pyrene concentrations and the numerical model were used to estimate the water boundary layer thickness so that the fractional equilibrium could be estimated. Using this fractional equilibrium and the diffusion coefficient for each analyte of interest, the numerically-modeled and measured concentrations for phenanthrene and benzo(a)pyrene were compared. This numerical model allows for the use of a small number of PRCs to be used to estimate boundary layer thickness so that the fractional equilibrium can be estimated for any analyte using appropriate diffusivity coefficients. The model demonstrates the large range of equilibrium times for chemicals measured in the laboratory in contrast to the longer times in the field. Finally, the importance of WBL thickness and molecular size on kinetics is demonstrated in both lab experiments and using the numerical model.

676 Kinetic passive sampling of nonpolar compounds in water: the effect of model choice on data quality

K. Booij, PaSOC

Passive sampling methods for nonpolar compounds are gaining maturity as routine tools for chemical monitoring in water. Equilibrium sampling is the most straightforward way to go, but when equilibrium cannot be attained on realistic time scales, kinetic sampling is the only option. Calibration of the in situ uptake kinetics is usually done by evaluating the dissipation of performance reference compounds (PRCs), using the PRC fractions that remain in the sampler after exposure, relative to unexposed samplers. Mechanistic models are available to calibrate the in situ uptake rates and to calculate aqueous concentrations. Next to these mechanistic models, some empirical models are being used. One of these empirical models is based on estimating the degree of equilibrium for all analytes of interest from a linear plot of retained PRC fractions versus log K_{ow}. With another empirical model, in situ sampling rates (R_s) are calculated for all individual PRCs, followed by evaluating the R_s of the target analytes from a linear regression of log R_s versus log K_{ow}. Using PRC data from field exposed samplers, it is illustrated that these empirical models may be acceptable in some cases, and in other cases may result in loss of trueness and/or precision, or even loss of data for some compounds.

677 Simulated sampling rates across the Global Atmospheric Passive Sampling (GAPS) Network

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Passive air samplers equipped with polyurethane foam media (PAS-PUF) are important tools for measuring atmospheric concentrations of persistent organic pollutants, but accurate and consistent determination of compound-specific effective sampling volumes for PAS-PUF deployments has been limited by the expense and uncertainty of sampler calibration and depuration approaches. Here, we evaluate global performance for compound-specific sampling volumes in a process-based hourly model using five semi-volatile depuration compounds and 82 samples collected by the Global Atmospheric Passive Sampling (GAPS) Network in 2006-2007. We apply the model using observed local meteorology from the Integrated Surface Database and calibrated for global use from the GAPS database. We evaluate the global spatial and seasonal distribution of depuration-based and simulated PAS-PUF sampling rates, quantify global predictive performance, and identify future research directions and operational approaches for refining estimates of PAS-PUF sampling rates. Results support the global operational use of simulated sampling rates specific to each site, deployment, and semi-volatile compound.

678 Gas-Particle Partitioning and Passive Sampler Calibration for an Expanded List of PAHs from Urban and Rural Ambient Air Sampling

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The extent of intra-urban PAH concentration variability is poorly known in the Twin Cities metro area and elsewhere. Active ambient air sampling requires electricity, security, and is cost prohibitive for finer spatial scale monitoring. Passive air sampling allows a larger number of samplers to be deployed within an area of interest, as they can be attached to any tree or utility pole (avoiding treated wood because of potential treatment contamination). Passive air samplers, however, require calibration using either active air sampling or depuration compounds. Our study calibrated passive mass loadings to active air sampling results by the calculation of an air sampling rate (Armitage 2013, Zhang and Wani 2012, Motelay-Massei 2005). An analysis of gas-particle partitioning, results below detection limits, and a sensitivity analysis of the summarization groups for active samplers (season, study year, study location, specific sites) was required in order to calculate air sampling rates that were both reflective of seasonality and stable enough to be useful for the entire study. Gas-particle partitioning was in keeping with previous studies and theoretical considerations. PAHs up to a molecular weight (MW) of approximately 166g/mole were found in the gas phase, while PAHs with MW between 166g/mole and 253g/mole partitioned between the gas and particle phases depending on ambient temperature and the chemical and physical characteristics of the PAH. Higher MW PAHs were found mainly in the particle phase. Partitioning coefficients were related to sub-cooled vapor pressures and octanol-air partitioning coefficients. There were several PAHs detected on the passive samplers (e.g. Benzo[a]pyrene, Benzo[e]pyrene, Benzo[j+k]fluoranthene) that were found exclusively in the particle phase in the active samplers. This finding could imply that the passive samplers collected some particles. Based on this finding we chose to use total (gas plus particle phase) active concentrations to calibrate passive samples. The air sampling rates for most PAHs were between 0.5-2.5m^3/day, but Dibenzothiophene sulfone was a clear outlier with sampling rates around 10m^3/day. We did not see a consistent relationship for all PAHs between sampling rate and ambient temperature. We did see a decrease in sampling rates with increasing molecular weight, possibly due to lower particulate sampling efficiencies in these samplers.

679 The potential role of mechanistic models for interpreting passive air sampling data for semi-volatile organic chemicals

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Passive air sampling is now well-established in the contaminant monitoring community and there are a variety of passive air sampler (PAS) designs described in the literature. Although PAS can differ with respect to sampler material (e.g., polyurethane foam, XAD resin, activated carbon felt, polyethylene, polydimethylsiloxane) and housing (e.g., none, 'flying saucer', cylindrical), the uptake and depuration kinetics are typically assumed to conform to the same theoretical model (Whitman Two-Film Theory). However, as the old aphorism goes, "All models are wrong but some are useful". Potential errors inherent to any modeling approach relate not only to the validity of the assumptions made (e.g., air-side resistance dominates exchange) but also the reliability of required input parameters (e.g., sampler-air partition coefficients and their temperature-dependence). The main objective of this presentation is to examine the underlying assumptions adopted in the PAS community with respect to uptake and depuration kinetics of semi-volatile organic chemicals (SVOCs) using the modeling tool developed within our research group (PAS-SIM). Insights from previous model evaluations using concurrent passive XAD-2 and active air sampling data are highlighted along with simulations of depuration compounds under different environmental conditions (e.g., temperature profiles and wind speeds). These model applications are used to discuss the advantages and disadvantages of applying mathematical tools to convert the mass of chemical accumulated on a PAS during deployment to air concentrations and also to identify key data gaps and research needs in this area.

Chemical and Microbial Environmental Health Threats Associated with Disasters

680 Regional Variability in Bed-sediment Concentrations of Wastewater Compounds, Hormones and PAHs for New York and New Jersey Impacted by Hurricane Sandy

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Bed sediment samples from 79 coastal New York and New Jersey, USA sites were analyzed for 75 compounds including wastewater associated contaminants, PAHs, and other organic compounds to assess the post-Hurricane Sandy distribution of organic contaminants among six regions. These results provide the first assessment of wastewater compounds, hormones, and PAHs in bed sediment for this region. Concentrations of most wastewater contaminants and PAHs were highest in the most developed region (Upper Harbor/Newark Bay, UHNB) and reflected the wastewater inputs to this area. Although the lack of pre-Hurricane Sandy data for most of these compounds make it impossible to assess the effect of the storm on wastewater contaminant concentrations, PAH concentrations in the UHNB region reflect pre-Hurricane Sandy conditions in this region. Lower hormone concentrations than predicted by the total organic carbon relation occurred in UHNB samples, suggesting that hormones are being degraded in the UHNB region.

681 Assessing Sediment Toxicity Predicted on the Basis of Sediment Chemistry in Barnegat Bay, New Jersey, Pre- and Post-Hurricane Sandy

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Hurricane Sandy made landfall in Barnegat Bay, New Jersey, October, 29, 2012, damaging shorelines and infrastructure. Estuarine sediment chemistry and toxicity were investigated pre- and post-event using samples collected at the same locations to evaluate potential environmental health impacts and to establish post-event baseline sediment-quality conditions. Trace element concentrations increased throughout the Bay up to two orders of magnitude, consistent with a northward redistribution of silt based on sediment transport modeling results. Conversely, there was a loss of organic compounds, clay, and organic carbon, consistent with sediment winnowing and transport. The number of sites exceeding sediment quality guidance levels, developed by Long and Morgan (1990), for trace elements tripled post-Sandy (most frequent, mercury and arsenic), and at one site, concentration of zinc exceeded the Effects Range-Medium level at which adverse biological effects have been noted in 50 percent of cases. Criteria for assessing sediment contaminants by region used consistently with the U.S. Environmental Protection Agency's (EPA) 2010 National Coastal Conditions Assessment indicated a change in sediment quality from good to fair post-Sandy. Potential toxic effects based on concentrations of sediment-borne contaminants in the Bay collected pre- and post-Sandy were assessed with EPA's Logistic Regression Model (LRM) developed for the test marine amphipod Ampelisca abdita. Measured sediment toxicity pre- and post-Sandy was the same for most sites and did not show statistically significant difference (paired-t test). At the site with the greatest relative increase for a suite of trace elements, survival rate of the test estuarine amphipod (Leptocheirus plumulosus) decreased, but survival rate did not decrease at the site with the large increase in zinc concentration. Predicted amphipod survival was not statistically significantly different pre- and post-Sandy, though the maximum calculated LRM values (used in computing survival rates) showed statistically significant increase post-Sandy corresponding with the increase in trace element concentrations. This study was possible because of the comprehensive baseline data enabling the evaluation of storm-derived changes in sediment quality, which can allow for similar evaluations for future events. Uncertainty in predicting potential toxic effects indicates greater understanding of factors affecting sediment toxicity is needed.

682 The US Geological Survey's Sediment-bound Contaminant Resiliency and Response Strategy: A decision support tool for evaluating contaminant hazards

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Coastal communities are uniquely vulnerable to sea-level rise and severe storms such as hurricanes. These events enhance the dispersion and concentration of natural and anthropogenic chemicals and pathogenic microorganisms, which could adversely impact the health and resilience of coastal communities and ecosystems in coming years. The U.S. Geological Survey (USGS) has developed the Sediment-bound Contaminant Resiliency and Response (SCoRR) strategy to define baseline and post-event sediment-bound environmental health stressors (see toxics.usgs.gov/scorr). A tiered, multi-metric approach will be used to: (a) identify and map contaminant sources and potential exposure pathways for human and ecological receptors, (b) define the baseline mixtures of environmental health stressors present in sediments and correlations of relevance, (c) document post-event changes in EH stressors present in sediments, and (d) establish and apply metrics to quantify changes in coastal resilience associated with sediment-bound contaminants. Integration of this information provides a means to better assess the baseline status of a complex system and the significance of changes in contaminant hazards due to storm-induced (episodic) and sea-level rise (incremental) disturbances. This talk describes the construction of a decision support tool to identify locations vulnerable to contaminants that may be mobilized by coastal storms. The support tool is designed to accommodate a broad array of geologic, land-use, and climatic variables and utilizes public, nationally available data sources to define contaminant sources and storm vulnerabilities. By employing a flexible and adaptable strategy built upon publically available data, the method can readily be applied to other site selection or landscape evaluation efforts. Examples from the SCoRR pilot study, key limitations, and future applications will be discussed in addition to ongoing method developments to accommodate non-coastal disaster scenarios and more refined contaminant definitions.

683 Organic Contaminant Screening and Storm-Derived Change: The US Geological Survey's Sediment-bound Contaminant Resiliency and Response Strategy

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With well over 100 million organic chemicals known, it is unfeasible to identify and quantitatively monitor all contaminants with potential environmental health implications. Traditional analytical approaches have proven to be expensive, time-intensive, and cumbersome. Adaptive management practices require timely data of sufficient quality to engage the policy and implementation process designed to guide restoration activities, assess environmental health changes, and support improvements in ecosystem services. In response, the U.S. Geological Survey is testing recently developed analytical methods for organic contaminants as part of the Sediment-bound Contaminant Resiliency and Response Strategy (SCoRR) project. SCoRR focuses on changes in sediment quality at targeted sights in northeastern U.S. coastal counties impacted by a 2015 storm. Screening measurements were conducted by protein phosphatase inhibition, fourier-transform infrared spectroscopy, and liquid chromatography with in-line ultraviolet/visible and fluorescence spectrometers and time-of-flight mass spectrometry. Converging lines of evidence approaches will be discussed using chemometrics to guide screening evaluation of results in the context of storm-derived change and environmental health.

684 Health risks in coastal flood of Río de la Plata

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Diseases are considered “epidemics” when the amount of affected exceeds the usual number of expected cases and an “outbreak” when an epidemic occurs in a small geographic area and for a short time. According to the Ministry of Health of the Nation among the phenomena associated with epidemics and outbreaks that have the greatest potential risk in our country they are: cholera, dengue, chikungunya fever and flu. In addition, “emerging diseases” occur when environmental changes favor the increase of pathogens and vectors, therefore increases the risk on the human population. Environmental factors that increase the risk of epidemic diseases may be mentioned environmental degradation (such as water pollution, garbage dumps and lack of hygiene), lack of potable water and sewage (favors pollution well water) and floods, situations that are associated with the increase in communicable diseases. In coastal areas affected by floods, the overflow of contaminated water, waterlogging and the backwaters favor migration of rodents (reservoirs of diseases) and breeding of mosquitoes and other vectors, provocateurs malaria, dengue and other vector-borne diseases . Furthermore contact with contaminated water, increasing cases of tetanus, hepatitis A, cholera, influenza, and pneumonia and other diseases caused by cold. This project addresses the health risks associated with exposure to flood events in coastal cities of River Plate, in order to provide guidelines for urban environmental management pollution. a selection of built-up coastal areas Quilmes, Berazategui, Ensenada, Berisso, Magdalena and Punta Indio was made and exposed to frequent occurrence of floods and overflows of wastewater. a characterization of the negative health effects on each selected site was made. Majoritarian diseases in the study area were: lung diseases or respiratory problems such as pneumonia, bronchitis, bronchiolitis, etc. and turn digestive system diseases such as gastritis, gastroenteritis, diarrhea, pain pelvic and certain diseases of infectious or parasitic origin.

685 Review of current literature of cardiotoxicity of oil to early life stages of fish for use in Natural Resource Damage Assessments

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After a release of contaminants, a Natural Resource Damage Assessment (NRDA) may be conducted to estimate the magnitude of injuries to natural resources, as well as the best options for restoration. Assessments for oil spills in the aquatic environment typically consider aquatic injuries related to phototoxic and narcotic effects on aquatic organisms, such as fish. Cardiotoxicity has recently emerged as an additional, potentially important mode of toxicity for Polycyclic Aromatic Hydrocarbons (PAHs) in early life stage (ELS) of fish. Suspected cardiotoxic effects, such as gross malformations, including pericardial and yolk sac edema, cranial facial malformations, and dorsal curvature, have been reported in fish species after exposure to PAHs. However, the repercussion of cardiotoxic effects is uncertain, as the reversibility of effects is possible and the link between laboratory observations to service losses relative to field populations is unclear. Additionally, available studies have significant limitations for use in a NRDA, including limited available field data to verify the effect beyond the laboratory, uncertain extrapolation to species of interest, and a lack of accurate information on the duration, concentration, and chemical composition of oil exposure. Additional limitations include uncertainty in the developmental windows of exposure for various species, inconsistent approach for calculating exposure of cardiotoxic PAHs, incomplete dose-response relationships reported in the literature, and insufficient data on long-term effects from cardiotoxicity. Future studies should further refine the cardiotoxic potential for different PAHs, verify that effects observed in the laboratory are observed in the field following exposure, and determine whether cardiotoxicity results in demonstrable long-term effects to individual fish and populations.

686 Young of the year bluefish as a bioindicator of estuarine health: Establishing a new baseline for persistent organic pollutants after Hurricane Sandy

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Atlantic coastal bays of the United States are essential habitat for young-of-year (YOY) bluefish (Pomatomus saltatrix). Their residence in these estuaries during critical-life stages, high lipid content, and piscivory make YOY bluefish an ideal bioindicator species for evaluating estuarine health. Hurricane Sandy made landfall in late October 2012, causing widespread physical, environmental, social and economic impacts to much of coastal New Jersey and New York. Individual whole YOY bluefish from four estuaries (2-3 sites/estuary) impacted by the storm were collected in August 2013, analyzed for a suite of persistent organic pollutants (POPs) including polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs) and organochlorine pesticides (OCPs) and evaluated using histological health metrics. Contaminant body burdens were similar between individuals collected from a single estuary and no site specific capture location differences were observed. The contaminant residue concentrations in the YOY bluefish reflected a broad-scale, integrated portrayal of the nursery ground in a given estuary, rather than a localized snapshot of the capture location (site), more commonly observed in resident species. Regionally, concentrations in YOY bluefish differed by estuary and concentrations for many POPs decreased or were similar to those observed prior to the hurricane. Prevalence of the ecto-parasitic gill isopod (Lironeca ovalis) on YOY bluefish varied by estuary and no relationships between contaminants and lesions were observed. The gill isopods removed from Jamaica Bay bluefish were preserved, homogenized and analyzed for PCBs, PBDEs and OCPs and this study was the first to report the presence of POPs in this common parasite. The co-existence of parasitic infections and contaminant exposure has various impacts on accumulation of hydrophobic contaminants in the host and in some instances the parasites are able to accumulate at a higher rate than their host making them potentially a better indicator of exposure. Contaminant information gained from bluefish and/or their parasitic isopods could be an effective way to examine an entire estuary rather than a single point location from multiple trophic levels. Fish health assessments in combination with contaminant analysis should also be included in regional monitoring programs to determine the potential consequences of residing and feeding in urbanized estuaries even for short periods of time.

687 Impact of Hurricane Sandy on PAH levels and oncogenes in Atlantic menhaden

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The impact of Hurricane Sandy (October 29, 2012) on PAH body burdens and genetic polymorphisms in oncogenes was investigated in adult Atlantic menhaden (Brevoortia tyrannus) collected along the NJ coast. Collections were made in August, September and/or October of 2011, 2012 and 2013. PAHs were monitored in raw fish oil using excitation-emission matrix spectroscopy (EEMs). Genetic polymorphisms were monitored using PCR primer sets designed to amplify V-Rel and C-Raf. The presence and absence of bands on agarose gels was used to determine the degree of polymorphisms. Primer sets were designed from an Atlantic menhaden transcriptome and verified by cloning. Results for

EEMS showed that raw fish oils had relatively high levels of high molecular weight, PAH-like compounds (173 to 24,421 ng/mL) compared to values reported for bile in other species. Concentrations and EEMs profiles varied by collection; however, collection ship, month, year and fish size did not account for the data. Preliminary data for oncogenes showed variation in polymorphisms by collection. Overall, Superstorm Sandy did not alter body burdens of PAHs in raw fish oil of menhaden.

Radionuclides in the Environment, Including Accumulation in Biota

688 Environmental radioactivity and public anxiety

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The disaster that befell the Fukushima nuclear power plant in March 2011, including the record release of radionuclides into the ocean, resulted in the expected radioactive contamination of water, sediments, and biota. The highest levels of radionuclides released into the Pacific were found closest to the source, that is, within the Fukushima prefecture. Radioactivity levels decreased markedly with distance from the source and greatly over time, reflecting radioactive decay (especially for ^{131}I) and dilution and dispersion by currents in the Pacific Ocean. Nevertheless, detectable levels of radiocesium were clearly evident in diverse marine fauna, ranging from small zooplankton to large apex predators, and indeed can still be measured, particularly in benthic fauna. Further, bluefin tuna which spawn in the western Pacific concentrated Cs isotopes prior to their migration across the Pacific to the eastern Pacific where we measured tissue radioactivities above the natural background. Although radioactivity levels were orders of magnitude below levels known to elicit any known toxic effects in fish or in human seafood consumers, there was a remarkable public outcry and widespread anxiety. Part of this anxiety may be due to the fact that radioactivity was spreading far afield, being visited on US shores, and in popular seafood items, no less. This disconnect between actual and perceived risks often accompanies environmental releases of radionuclides, and reflects a broad-based fear and ignorance of radioactivity. This anxiety regarding environmental radioactivity may well be a legacy of cold war era anxieties, as depicted in lurid science fiction movies or even more serious films that displayed the potential consequences of nuclear releases. The anxiety is also attributable to the invisible nature of the threat, the terrible consequences of exposure to high radioactivity levels, and the considerable uncertainties of the impacts, if any, of exposures to very low radioactivity levels. A plea will be made to encourage renewed education and training involving environmental radioactivity so that the US can return to a position of leadership and competence it once had some fifty years ago in this field. A more informed citizenry would also be better able to soberly judge the risks involved in nuclear matters and voice rational opinions on future energy policies.

689 Are Sediments a Source of Fukushima Radiocesium for Marine Fauna in Coastal Japan?

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The Fukushima nuclear power plant accident in 2011 resulted in the largest accidental release of artificial radionuclides into the world's oceans. Among the fission products released in large quantities, ^{137}Cs has the greatest potential for long-term impacts on marine biota and human consumers of seafood. Marine animals, seawater and sediment near Fukushima, Japan have become contaminated with ^{137}Cs released from the damaged nuclear power plant. Radiocesium concentrations in some benthic fauna declined more slowly than in pelagic fish in the same region. A year after the accident, ^{137}Cs concentrations in both surface and bottom water declined sharply and remained very low; while ^{137}Cs concentrations in coastal sediment remained rather high and declined very gradually over time. We tested the hypothesis that benthic fish remained more contaminated due to the bioavailability of radiocesium in sediments. Desorption of Cs from sediments followed a concentration gradient from sediment surface to overlying seawater. We assessed the bioavailability of dissolved and sediment-bound Cs for deposit-feeding polychaetes, and its subsequent transfer to crabs and fish. Laboratory experiments demonstrated that the assimilation efficiency of ^{137}Cs was 16% in polychaetes ingesting Fukushima sediment, up to 54.5% in crabs ingesting polychaetes, and about 80% in fish ingesting worms. Thus, deposit feeding invertebrates and their predators may acquire Cs from contaminated sediment and introduce it into the benthic food chain. In addition, all animals acquired Cs from the aqueous phase, accounting for up to 65% of their total body burden. Loss rate constants following dietary uptake were 45% d^{-1}, 14% d^{-1}, and 5% d^{-1} for polychaetes, crabs, and fish; rate constants following aqueous uptake were 20% d^{-1} for polychaetes, 10% d^{-1} for crabs, and 6% d^{-1} for fish, respectively. A bioaccumulation model indicated that the transfer factors of Cs from sediments and the trophic transfer factors from worms to predators were about 1. Our results are consistent with the idea that sediments can be an important source of Cs for benthic food chains and help explain why bottom fish remained more contaminated than pelagic fish in Japanese coastal waters after the nuclear accident.

690 Environmental fate of radiocesium in an aquatic and semi-aquatic food web on the Savannah River Site

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Although biomagnification of radiocesium (^{137}Cs) has been reported in aquatic ecosystems, previous reserch has been limited to select trophic linkages and few studies have included a comprehensive survey of fauna associated with aquatic, semi-aquatic, and terrestrial habitats within a single study framework. Furthermore, few studies have examined the effect of ontogenetic shift in foraging strategy and diet on radionuclide burdens between juvenile and adult organisms. The objective of this study is to advance our understanding of the dynamics of ^{137}Cs accumulation within food webs by quantifying ^{137}Cs concentrations across a wide range of biota found within contaminated wetland ecosystems on the U.S. Department of Energy's Savannah River Site, and aims to determine if changes in life history, specifically in cottonmouths (Agkistrodon piscivorus) and bullfrogs (Lithobates catesbeianus), contribute to differences in ^{137}Cs burdens between juvenile and adult individuals. Specimens representing a range of different taxa, including invertebrates, fish, amphibians, reptiles, and mammals, were collected from the contaminated R-Canal and Pond A ecosystems on the Savannah River Site, and tested to quatify ^{137}Cs concentrations in their body tissues. Results show that ^{137}Cs concentrations are not always greater in organisms representing higher trophic positions within contaminated ecosystems. Additionally, the data shows significant variation in ^{137}Cs burdens between juvenile and adult life stages in cottonmouths and bullfrogs. This suggests that ^{137}Cs does not undergo biomagnification when examining trophic level increase in aquatic and semi-aquatic/terrestrial organisms, and that ontogenesis between juvenile and adult life stages can influence ^{137}Cs accumulation in exposed individuals.

691 Effects of chronic radiation exposure on carnivores from the Chernobyl Exclusion Zone

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Nuclear energy production is increasing globally, yet long term ecological implications of radiation exposure from normal industry practices or accidents such as Chernobyl or Fukushima are unclear. Acute exposure to radiation is widely recognized to cause morbidity and mortality in wildlife as well as have sub-lethal influences such as effects on immune response, with low-dose stimulation and high-dose suppression of immunity. However, robust information is lacking regarding the impacts of chronic radiation exposure. The Chernobyl Exclusion Zone (CEZ, evacuated and excluded since 1986) offers an ideal model system to investigate these long-term chronic effects of radiation exposure on wildlife populations. The CEZ harbors a heterogeneous landscape of radiation levels (40 – >7,500 KBq/m^2) and several carnivore species. Carnivores are at higher risk for accumulation of contaminants due to their high trophic level and long life span, and thus provide a good model system. Our research provides the first comprehensive study examining carnivore density, distribution, genetic characterization, and prevalence of parasites and disease as a function of radiation exposure. During fall 2014 and spring of 2015, we collected data on carnivores within the CEZ. We found that radiation levels do not influence the distribution of any of the mammal species we investigated. However, our preliminary data show a trend of higher gastrointestinal parasite loads of Coccidia and Alaria species in individuals exposed to higher levels of radiation, which may be indicative of complex sub-lethal effects experienced by chronically exposed individuals. Disease prevalence is affected by population density and we are currently using genetic mark recapture methods to estimate densities of two of the most commonly occurring carnivores in the CEZ, the gray wolf (Canis lupus) and raccoon dog (Nyctereutes procyonoides. When complete we can investigate parasite and disease prevalence as a function of radiation exposure, radiocesium body burden counts, population density, and genetic characteristics. Addressing individual health through parasite and disease prevalence provides insight into mechanisms affecting health of chronically exposed carnivores in the CEZ. Further work will incorporate these and other results into population based analyses to provide insight into the population level implications of chronic radiation contamination exposure.

692 Sources of radioactive cesium to marine biota off Fukushima

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Following the radioactive spill from the damaged nuclear power plant in Fukushima Prefecture in Japan in March and April of 2011, the levels of ^{134}Cs and ^{137}Cs in seawater and various marine fish caught off Fukushima steadily declined. A year after the Fukushima disaster, radioactive Cs (i.e. ^{134}Cs + ^{13}7Cs) in most of these fish remained elevated above human advisory of 100 Bq kg^{-1} wet weight. Moreover, coastal sediments off Fukushima were found to contain higher levels of radioactive Cs than in other coastal regions off Japan, suggesting that these sediments acted as a repository for the Fukushima Cs. We hypothesized that these contaminated sediments could serve as a source of radiocesium to organisms that are part of the benthic food web in that area. To evaluate the relative importance of Cs sources Cs (i.e. sediment vs. seawater) for marine organisms from that region, we collected biota representative of the pelagic, benthic and coupled benthic-pelagic food webs and different trophic levels during 3 cruises aboard Japanese research vessels from May of 2013 to September of 2014. We measured levels of radioactive Cs for muscle tissues of individual fish species or for bulk biomass for planktonic organisms to assure sufficient mass for radioanalysis. The ratios of stable isotopes of C, N and S i.e. δ^{15}N, δ^{13}C and δ^{34}S were also measured to apply to Bayesian mixing modelling, which served to identify trophic linkages (i.e. benthic, pelagic or coupled) for these biota. Based on modelling, which was largely influenced by the δ^{34}S values (< 19‰ for benthic organisms) we confirmed that fish with the highest radiocesium activities relied mostly on benthic dietary sources. The data suggest that Cs accumulated by these fish was remobilized from sediments off Fukushima and was transferred from sediment to bottom dwelling invertebrates i.e. meiofauna and macrofauna, which were subsequently eaten by various benthic fish such as cod and flatfish species. Thus, sediments may serve as both a sink and a source for radionuclides released from Fukushima.

693 The Influence of Residence Time on Uptake of Radiocesium by Waterfowl on a Contaminated Reservoir on the Savannah River Site

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Former nuclear reactor cooling reservoirs on the U.S. Department of Energy's Savannah River Site (SRS) historically received relatively low releases of radiocesium in the mid- to late 1900s. Migratory waterfowl visiting these reservoirs present a special concern for potential exposure of offsite sport hunters to radiocesium by consumption of contaminated meat. Recent collections of free-ranging waterfowl suggested that the Pond B reservoir has higher radiocesium concentrations than other SRS reservoirs and that American coots (Fulica americana) had higher levels of radiocesium than diving ducks. Historical monitoring on SRS has generally incolved collections of free-ranging watefowl, with no knowledge of residence time on the contaminated reservoirs. To examine the influence of residence time on radiocesium uptake, we released wild-captured coots and ring-necked ducks (Aythya collaris), whose flight capabilities were restricted by scissoring flight feathers, on a radiocesium-contaminated reservoir (Pond B) and then made lethal collections of both species after known residence times ranging from 32 -173 days. Both species accumulated radiocesium, with ring-necks accumulating slightly higher concentrations, but acheived asymptotic levels of whole body radiocesium over different periods with coots taking ~30 days and ring-necks taking ~75 days. Radiocesium levels in a small sample of both species appeared to decline > 90 days post-release. Differences in radiocesium levels between species are likely due to dietary differences and the possible late-season decline may be due to seasonal differences in diet or radiocesium bioavailability.

694 Uptake and dosimetric modeling of Tc-99, Np-237, and U-238 in the grass species Andropogon Virginicus

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The purpose of this work is to quantify the uptake of ^{99}Tc, ^{237}Np, ^{238}U into the grass speciesAndropogon Virginicus as well as to develop and compare associated dosimetric models. These risk driving radionuclides were chosen for their wide range of biogeochemical behaviors, and A. virginicus was selected as it is a common ground covering in the Southeastern United States. Hydroponic experiments in a laboratory setting were used to determine the potential for A. virginicus to physically take up the nuclides, giving insight into the mechanisms that may cause previously observed upward soil column migration at the Savannah River Site. The hydroponic studies consisted of growing established A. virginicus specimens in Hoagland nutrient solution containing the aforementioned nuclides under a 12 hour light cycle with plants being harvested in groups of four at three time points post exposure. The experiment was repeated at the seedling stage in to investigate the influence of plant life stage on uptake. Analysis of nutrient solution and plant material was conducted through inductively coupled plasma mass spectrometry, liquid scintillation counting and autoradiography to compare uptake and translocation between the three different exposure times. Shoot concentration ratios for ^{99}Tc ranged from 6 to 17 L kg^{-1}, for ^{237}Np ranged from0.2 to 0.9 L kg^{-1}, and for ^{238}U ranged from 1 to 7 L kg^{-1}. Dosimetric models were developed for A. virginicus utilizing stylized representation of plant organs (shoots and roots), with preliminary work in voxel modeling. Dose conversion factors (μGy d^{-1} per Bq kg^{-1}) were calculated using Monte Carlo transport codes. Results are compared to existing ellipsoidal models within the ERICA tool and RESRAD-BIOTA; ellipsoidal models provide a conservative estimate of dose rates to the whole plant, yet in many instances a plant's structure is not well-represented by an ellipsoid. Comparative absorbed dose rates, determined by combining the uptake results with the dosimetric model, are reported for the laboratory-grown A. virginicus. In general, the development of more comprehensive, yet practicable, dosimetric models of non-human biota allows for a greater ability to quantify potential dose-effect relationships and evaluate potential environmental radioecological impacts.

695 Monitoring elemental concentrations and radioactivity in biological samples at uranium mines in the Grand Canyon watershed: The Kanab North site

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The U.S. Geological Survey and its cooperators are currently engaged in biological and chemical monitoring of reference and mining sites in pre-, post- and active-uranium ore production stages in the Grand Canyon watershed (Arizona, USA). The present paper describes the results of chemical (e.g., nickel, copper, zinc, arsenic, selenium, lead, thallium, uranium) and radiological analyses of animal (brush mice, deer mice) and plant (salt cedar, sage brush, needle and thread grass) tissues collected at the Kanab North Mine site. The Kanab North Mine was mined for uranium ore from 1988 to 1990, and then placed on standby in 1992. Reclamation of the site began in 2013, and is scheduled for completion in fall 2016. However, contamination, including elevated uranium concentrations in plants and dust, still exists at the Kanab North site; and the lengthy standby status of the mine likely contributed to low-level chronic exposures of the biota to weathered ore, radioactivity, and related chemical constituents. The results for Kanab North will be compared to data obtained for animal and plant tissues at the Canyon Mine, a pre-mining site currently undergoing shaft sinking, and at a nearby non-mineralized reference site; concentration results will be compared to literature-based toxicity thresholds. These comparisons will help inform on the ecological effects of uranium mining in the Grand Canyon watershed.

Methods and Applications of Non-targeted Mass Spectrometry for Environmental Characterization

696 High-resolution metabolomics for sequencing the human exposome

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Over the course of a lifetime, humans are exposed to a wide range of chemicals from multiple sources. Surveys using targeted biomonitoring approaches have considerably advanced understanding of the distribution and occurrence of select exposure hazards; however, analytical targets are often limited and measure in large populations is not cost-effective. To better understand the role of environmental exposures in disease, there is a need for analytical methodologies that provide systematic measure of the human exposome. In an effort to accomplish this goal, we have developed a high-resolution metabolic phenotyping strategy utilizing complimentary measures by gas-chromatography (GC) and liquid-chromatography (LC) with ultra-high resolution mass spectrometer (MS) detection. Semi-volatile chemicals were measured by GC with detection by Orbitrap MS following sample preparation using a modified quick, easy, cheap, effective, rugged and safe (QuECHERS) method optimized for small (< 200 µL) volumes of plasma. Detected chemical classes included organochlorine pesticides, polychlorinated biphenyls and brominated flame retardants, in addition to unknown spectra exhibiting fragments consistent with other organohalogen compounds. Non-volatile species were measured in 65 µL of plasma using triplicate analysis with C_{18} and HILIC stationary phases interfaced to an Orbitrap MS. Plasma proteins were first precipitated by treating 65 µL of plasma with acetonitrile and the supernatant was analyzed in triplicate using C_{18} and HILIC column stationary phases. Following data extraction and alignment, over 10,000 mass-to-charge (m/z) ions were detected. Ions were annotated using isotopic pairing, accurate mass matches to chemical databases and comparison to authentic reference standards. Identified species included chemicals arising from commercial products, endogenous metabolism and dietary sources, with >50% of the m/z ions providing no matches in the chemical databases. Using the combined analytical capabilities of GC and LC with high-resolution MS and simplified sample preparation, it is possible to provide measures of a large number of environmental chemicals and endogenous metabolites in a relatively high-throughput and cost-effective manner. The resulting data can be used to estimate exposure in human populations, evaluate disease-exposure associations and provides systematic measures of environment and biological response for exposome research.

697 Development and Evaluation of Non-Targeted Screening Methods with the use of NIST Standard Reference Materials

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The creation of environment non-targeted screening protocols requires the development of multiple aspects of an analytical method, including sample preparation, instrumental analysis, and data analysis. The impact of each one of these steps can be significant to the outcome of a non-targeted screening analysis of an environmental material. For these reasons, environmental materials that are abundant, homogeneous, and long-term stable are extremely valuable as test materials to develop non-targeted screening methods and to evaluate the performance of the developed methods. Standard Reference Materials (SRMs), as developed by the National Institute of Standards and Technology (NIST), are excellent resources for the development and testing of non-targeted screening protocols. A general overview of NIST SRMs will be presented, in regards to the creation and testing procedures used to create stable, homogenous, and relevant environmental materials. In the past few years, multiple researchers within and outside of NIST have used NIST SRMs (such as SRM 2585 Organic Contaminants in House Dust) to test out newly developed non-targeted screening protocols. Some examples of the use of NIST SRMs for non-targeted screening method development will be discussed, including successful compound identifications made using GCxGC-MS and LC(xLC)-HRMS techniques. Using NIST SRMs, multidimensional chromatography paired with mass spectrometry provides enhanced identification of unknown compounds through "cleaner" mass spectra from better chromatographic resolution. Compounds including personal care products and flame retardants were successfully identified by newly-developed non-targeted screening methods. As reproducibility of LC-HRMS non-targeted screening methods can be a concern, new computational tools were developed to evaluate whole-method reproducibility and will be demonstrated as applied to SRM 2585. Finally, a new type of water reference material is in development for organic analysis of aqueous environmental samples using solid-phase media. Through the use of non-targeted screening methods, we were able to examine the nature of the new material and evaluate its use for targeted and non-targeted analysis.

698 Use of Chemometrics and Computational Chemistry in Non-Targeted and Targeted Analysis of PAH Transformation Products in Various Environmental Matrices

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Degradation of parent polycyclic aromatic hydrocarbons (PAHs) in the environment is well established and recently the transformation products formed have been shown to be more toxic than parent PAHs. The measurement of these transformation products is less frequent because, often, the identities of these products are unknown. Non-targeted approaches are increasingly used to measure PAH transformation products in various environmental matrices that were previously unidentified. In addition to that, previous research has shown that the formation of these transformation products can be predicted using computational chemistry, using the thermochemical properties of chemical compounds. For instance, using the density functional theory (DFT), the Gibbs free energy of the reaction pathway from a parent PAH to transformed products can be calculated. This unique capability can be a powerful tool that aids analytical chemists in determining the likeliest transformation products that will be formed in the environment. However, computational chemistry alone cannot differentiate which products will likely form when biological processes are involved in the degradation process, such as in the presence of microbial enzymes. Chemometrics can complement computational chemistry to help solve this issue. This technique uses statistical models and curated data in the literature to provide chemical information. The Pathway Prediction System (PPS) from the University of Minnesota and the Swiss Federal Institute of Aquatic Science and Technology, is an example of database that uses this approach and can provide the chemical structures formed from microbial biodegradation. Together, with analytical methods, both techniques may provide researchers with a priority list of transformation products that will likely to be formed based on their thermodynamic data, which is important in non-targeted analysis. Moreover, with the presence of open sourced computer programs, chemometrics and computational chemistry offer a unique perspective for non-targeted analysis that can be adopted by a variety of labs as the techniques are independent of specific analytical instrumentations.

699 Characterization of organic micropollutants in ballast water and their role in disinfection byproduct formation following oxidative treatment

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More than 10 billion gallons of seawater are transported globally each year during shipping activities. Ballast water has been identified as a major vector for the translocation of aquatic marine species, contributing to the spread of invasive species and pathogens. In an effort to mitigate ballast water impacts on marine ecosystems, the International Maritime Organization (IMO) has established the Ballast Water Management Convention in 2004. Pending regulations will require the installation of type-approved Ballast Water Management Systems (BWMS) in approximately 57,000 merchant ships globally, replacing and supplementing existing standards dictating open ocean tank water exchange. The majority of treatment systems use oxidizing agents, particularly chlorine, ozone or UV to kill invasive species or pathogens, generating halogenated disinfection byproducts (DBPs). The composition of DBPs is dependent on organic substrates present in the seawater, as well as environmental characteristics such as pH, salinity, temperature and total organic carbon. To better understand the chemical microenvironment of ship ballast tanks and the potential for DBP formation after treatment, we sampled ballast water from ships in the USA (east and west coast) and Singapore. In addition, test-scale and bench-scale BWMS systems were tested with several water sources differing in salinity, temperature, and organic carbon content to examine DBP production. Treated and untreated ballast water was characterized by HPLC with Orbitrap high resolution tandem mass spectrometry for both targeted and non-targeted polar micropollutants and DBPs, as well as for known volatile/semivolatile DBPs using GC-MS. Results indicate that micropollutant burdens and identities vary considerably according to ship location and water source, with complex polyethoxylated surfactants identified as a ubiquitous contaminant class. These and other organic micropollutants may form potentially toxic transformation products upon chemical or UV treatment for disinfection. Results of our measurements will be presented in context of implications for ballast water management for global shipping.

700 Non-targeted identification of contaminants in Southern California sentinel marine mammals

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Apex marine predators of the Southern California Bight face a complex combination of environmental stressors including exposure to anthropogenic contaminants. These animals also serve as effective indicators of marine pollution due to their high trophic position, longevity, and large blubber stores. We developed an innovative non-targeted analytical method to characterize known and unknown anthropogenic contaminants often missed by traditional targeted methods. Coincident with the identification of anthropogenic compounds was the identification of several classes of halogenated natural products. The analytical method utilized comprehensive two-dimensional gas chromatography coupled to time-of-flight mass spectrometry (GC×GC/TOF-MS) to generate a full inventory of halogenated organic compounds from archived blubber samples of five candidate marine mammal species: long-beaked common dolphins (Delphinus capensis), short-beaked common dolphins (Delphinus delphis), Risso's dolphins (Grampus griseus), California sea lions (Zalophus californianus), and harbor seals (Phoca vitulina). In addition to the instrumental method, data management was critical to establishing the contaminant inventory and required the development custom software. A software tool to automatically identify halogenated mass spectra improved the data processing efficiency and allowed for the relatively large sample set compared to previous projects. Additional software was used to assist with compound identificaiton, organize mass spectra and ancillary information, ensure the reproducibility of identifications, and provide a mechanism for sharing the data (a mass spectral library) with other researchers using a standard data format. Compound identifications were performed through manual interpretation of mass spectra, and automated searching of standard reference and custom mass spectral libraries. Identified chemicals were catalogued and exposure profiles for each species were generated based on contaminant abundance and frequency of occurrence. Optimal sentinel species for future environmental monitoring were identified based on the magnitude and multitude of accumulated compounds. This research ultimately aims to develop a streamlined and comprehensive approach for the discovery of emerging contaminants, investigate associations with health outcomes, and reinforce the importance of non-targeted analytical methods in informing environmental monitoring and assessment.

701 Chemical assessment of effects on invertebrates using high-resolution mass spectrometry

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The Cache Slough complex is an area of tidal sloughs in the Sacramento–San Joaquin River Delta of California (USA) and is an important habitat for endangered fish species (e.g., the endemic Delta Smelt). Existing pesticide data are sufficient to demonstrate a risk to aquatic invertebrates upon which several fish species depend. This study involved deployment of a sensitive amphipod species, Hyalella azteca, an important fish prey in the study area, combined with a complete chemical screening of water samples during two storm events in winter 2015/2016. The use of high-resolution mass spectrometry thereby allowed the screening for unknown chemicals. Water samples were concentrated by solid phase extraction. Non-polar pesticides were analyzed on an Agilent GC-QTOF-MS/MS. Negative chemical ionization (NCI) mode was selected for the target screening of pyrethroids and fipronil due to its high sensitivity. To identify further suspected pesticides without a reference standard, samples were measured in electron impact (EI) mode using an accurate mass spectral database containing 750 pesticides (Agilent Technologies). Polar chemicals were analyzed on an Agilent LC-QTOF-MS/MS using electrospray ionization (ESI) in both positive and negative mode. 27 target chemicals were selected to validate the method. Data were acquired with an All-Ions fragmentation method in order to carry out suspect screening using an accurate mass MS/MS database consisting of 1700 pesticides and related compounds. In addition, the samples were screened for a list of 1300 transformation products which were predicted by a biochemical pathway predictor (EAWAG-PPS). Finally, molecular features of all chromatograms were extracted to look at true unknowns in the sample. Features with significant differences between affected and non-affected samples were prioritized using Agilent Mass Profiler Professional (MPP) software. Over 120 different pesticides and transformation products could be detected at least once; 90 of them were found by the suspect screening. Many of the detected compounds correlated with toxicity toward H. azteca. The study clearly showed that classical targeted approaches would have missed a large part of the exposure. Stujdy results provide crucial information about chemicals that were the cause of observed toxicity to deployed H. azteca, which will represent a valuable resource for future watershed management toward the protection of the fragile delta ecosystem.

702 Suspect and Non-Target Screening of Organic Contaminants In Urban Aquatic Environments

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For issues of both ecosystem and human health, identifying toxicants and other important bioactive molecules in high priority samples that include complex mixtures of many different contaminants is a challenging analytical task. Here, we present the use of high resolution quadrupole time of flight mass spectrometry (QTOF LC-MS/MS) to characterize the occurrence and fate of otherwise uncharacterized trace organic contaminants in aquatic environments. To better understand and prioritize the contaminates of potentially high ecological risk, we have analyzed paired water-tissue samples from fish experiencing acute mortality when exposed to stormwater runoff to investigate contaminant uptake and metabolic processes, while isolating a subset of high interest detections for targeted investigation. Sample collection and processing, data reduction, quality assurance-quality control, and data analysis workflows are described. Additionally, we will describe the use of QTOF LC-MS/MS to understand contaminant transformations screening HRMS fragment and mass defect data to identify transformation products, focusing on the fate of endogenous and xenobiotic compounds in the wastewater effluent. For example, we tentatively identified adrenosterone, a chlorination product of glucocorticoid compound, in the wastewater influent with reference standard and MS/MS library. However, possible existence of adrenosterone isomers presents additional challenges in determination of adrenosterone in the wastewater especially when properties of isomers are similar. Such investigations clearly point to the importance of combining chemical analysis with biologically based tools that allow for a comprehensive and holistic analysis of contaminant occurrence and fate in aquatic systems, with particular emphasis on identifying contaminants with bioactive and pharmacological properties from a complex mixture.

703 Targeted Discovery of Disinfection Byproducts in Swimming Pools and Spas

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Swimming pools are treated with disinfectants to protect swimmers from pathogens and prevent illness. The water used to fill a swimming pool if from a municipal drinking water supply, is also often treated with disinfectants such as chlorine. Disinfectants will react with naturally occurring organic matter in water and, in the case of swimming pools, they can also react with chemicals introduced to the water by the swimmers themselves to produce byproducts that can be potentially harmful. It is important to treat water while minimizing the risk of disinfection byproducts (DBPs). One of the first steps is to chemically characterize the DBPs in swimming pools and hot tubs, very complex matrices, using discovery techniques since a lot of the contaminants are unknowns. Comprehensive two-dimensional gas chromatography high resolution time-of-flight mass spectrometry (GC×GC-HRTOFMS), was used for the tentative identification of “known unknowns” and “unknown unknowns” in swimming pool and hot tub water. The “known unknowns” were identified by library database searching deconvoluted spectra using LECO’s ChromaTOF software. The “unknown unknowns” were tentatively identified using a combination of EI and CI accurate mass data for chemical formulae determination and structural elucidation by leveraging the accurate mass fragments.

Mixtures: Exposure and Toxicity from Combinations of Stressors

704 Juvenile mite sensitivity and fitness reduction of successive mite generation after exposure to metals

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Metals like many other pollutants usually exist as mixtures in reality and toxicity data should be based on metal mixtures. Metals in mixtures could interact and the interactions could influence the end toxicity (synergism or antagonism) different from addition of individual toxicities (concentration addition). Metals persist in soil and thus, exposure to metals can have a multi-generational effect on soil organisms. This study assessed single metal and metal mixture toxicity of five metals on juvenile survival and fitness reduction in generations of two species of mites, Oppia nitens and Hypoaspis aculeifer. For the single metal tests, mites will be exposed to eleven concentrations of each metals in soil. For the mixture tests, mites will be exposed to the five metals in ten fixed-ratio rays and eight dose levels. The fixed ratio rays will be chosen based on contaminated site ratios and regulatory ratios. Juvenile survival will be assessed after relevant duration period for each species and its sensitivity will be compared to that of adult reproduction. Adult species will be exposed to single and metal mixtures and the control experiment will be adults not exposed to metal. The progeny from the experiment will be kept unexposed till the F3 generation. The F3 generation of the exposed and non-exposed mites will then be exposed to single and metal mixtures. The fitness will be assessed through differences in reproduction after metal stress as well as the performance of progeny in avoidance tests.

705 Predicting Sediment Chemical Mixture Toxicity from Marine Amphipod Mortality Data

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Sediment quality objectives in California are informed by multiple lines of evidence, including toxicological experiments exposing amphipods to dredged sediments containing mixtures of chemicals that are partly described from targeted chemical analysis. No individual sediment characteristic has been found to be strongly predictive of amphipod mortality, raising the possibility that toxicity arises from the joint effects of the mixture. In this analysis, we establish that interactions among sediment components can explain sediment mixture toxicity, and explore whether any subset of mixture components is especially predictive. Sediment toxicity data were provided by the Southern California Coastal Water Research Project. Grab samples were taken at 1322 locations in California bays and estuaries between 1995 and 2003 as part of multiple regional monitoring and sediment characterization studies. Toxicity assays were conducted using the estuarine amphipod Eohaustorius estuarius. Chemicals were measured by AAS, ICP-MS, CVAFS, GC-FID, GC-ECD, and GC-MS, LC/MS or GC/MS as appropriate. Canonical correlation analysis (CCA), exploring which vector of chemical predictors is most correlated with amphipod mortality, and gradient boosted models (GBM), a tree-based approach for exploring whether nonlinear conditional relationships hold additional predictive value for mortality (dichotomized at ≥60% of amphipods dead) were used to evaluate the mixture of chemicals in relation to amphipod mortality. The most informative sediment components were total organic carbon, lead, iron, cadmium, zinc, and 4,4 DDD from GBM, and zinc, 2-methynaphthalene, and 4,4 DDD from CCA. The canonical variate for sediment chemical mixture explained 30% of the variation in toxicity. The bootstrapped area-under-the-curve was 0.65 (95% CI=0.61, 0.68), indicating that the GBM was an informative classifier. The GBM suggested that a tree depth of 4 splits provided optimal prediction of amphipod mortality, implying that higher-order interactions between mixtures of chemicals hold additional predictive value. The toxicity of sediment mixtures appears to be partly explained by interactions between mixture components.

706 Inverse U-shape and non-monotonic dose-response for the toxicity of Bt biopesticide to D. magna

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Bacillus thuringiensis (BT) pesticides, such as DiPel ES, are amongst the worldwide most used to control caterpillars. BT, an entomopathogenic bacteria that produces endotoxins, causes effects described as highly specific to target insects and therefore it is used worldwide without environmental limits. The exposure of high concentrations of components of this biopesticide to non-target organisms results generally in negligible effects. This study investigated the toxic responses of the non-target organism Daphnia magna to waterborne DiPel ES, as the mixture used in the application (BT, its endotoxins, its exotoxins, its spores, formulation products, fermentation products) and in environmentally relevant levels (24 concentrations from 0.0025µL/L to 500µL/L). Neonates were 48h-exposed to DiPel ES for LC50 and EC50 estimation. D. magna 17-21 days old were similarly exposed for EC50 and biomarkers. The biomarkers evaluated (whole body) were body weight, global protein, chitobiase, catalase, glutathione reductase, glutathione S-transferase, and acetylcholinesterase. Particle size distribution in the exposure media was further determined by light scattering analyses.Dipel affected immobilisation and mortality of neonates ($p>0.001$, EC50=from 0.17 to 0.20µL/L, LC50=0.40 to 1.17µL/L). This toxicity is ~100,000 higher than announced by manufacturer. Similar sensibility was found for immobilisation of adult daphnids (EC50= from 0.17 to 0.29µL/L), without significant mortality. Mortality and immobilization endpoints displayed a biphasic and inverse U-shaped response. This clearly not monotonic exposure challenges the central assumption of ecotoxicology, i.e. that toxicity only increases with increases in the concentration of contaminants. All biomarkers but acetylcholinesterase were affected by DiPel ES exposure ($p< 0.05$), displaying multiphasic dose-responses. This suggests a mechanism of toxicity able to affect generally organs and systems of the whole organism. The main particle size in experimental media was compatible to bacteria spores, however a secondary particle size compatible to metabolic active BT, was observed when highest toxicity was occurring. Therefore, it is possible that chemical and physiological interactions resultant of different behavior of BT and DiPel ES components at various concentrations might be related to the observed non-monotonic effects. Further studies are required to isolate the cause of non-monotonicity of this BT biopesticide.

707 Mesocosm assessment of the effects of an organic ultraviolet filter mixture and elevated seawater temperature on corals

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Organic ultraviolet (UV) filters, active ingredients added to personal care products to protect skin from harmful effect caused by UV radiation, have been detected in surface waters at concentrations ranging from ng/L to µg/L levels. Previous studies reported that organic UV filters caused coral bleaching at low concentrations. Coral reefs are known to be one of the most biodiverse and important ecosystems on Earth, but reefs in many areas are facing multiple threats, including thermal stress due to global climate change. Despite the potential negative effects of organic UV filters on the health of corals, information is lacking about their toxicities. In addition, the simultaneous effects of organic UV filter exposure and thermal stress on corals also should be of concern. A 60-day mesocosm study was performed to test the effects of an organic UV filter mixture under general environmental concentrations and elevated temperature on coral nubbins [Seriatopora caliendrum (SC), Pocillopora damicornis (PD) and Montipora aequituberculata (MA)]. The mixture contained 200 ng/L benzophenone-3, benzophenone-4, benzophenone-1, benzophenone-8, octinoxate, amiloxate, enzacamene, avobenzene, ethylhexylsalicylate, homosalate, octocrylene, and octyl dimethyl-p-aminobenzoic acid. Treatments including (A) solvent control, (B) organic UV filters mixture alone, and (C) organic UV filters mixture + elevated temperature (30°C) were applied. Growth (n=4, Day 14, 30, 60), mortality, bleaching and polyp retraction (n=8, daily) were measured. Nubbins in treatment A appeared healthy throughout the exposure. Bleaching (37.5%) and death (12.5%) of SC in treatment B were observed. In treatment C, 100% bleaching and mortality of SC and 100% bleaching and 50% mortality of PD were found. Bleaching (50%) and thorough polyp retraction (100%) of MA nubbins in treatment C were also observed. Growth of SC in treatment B and of all three species in treatment C were significantly reduced. These results indicate that organic UV filters at environmental concentrations caused bleaching and death of vulnerable coral species, e.g. SC, while the combination of two conditions caused lethal effects to SC and PD. MA was found to be the most tolerant species under these conditions in our study. Future work will include measurements of gene expression [e.g. cytochrome P450, vitellogenin, superoxide dismutase (SOD)] and enzyme activities (e.g. SOD), and chemical analysis of tissue samples.

708 Variation in species sensitivity distribution obtained from ecotoxicity data of mixtures

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Species sensitivity distributions (SSDs) are increasingly used in ecological risk assessment procedures and formulation of water quality guidelines. The usefulness of SSD analyses is likely to depend, at least in part, upon the quality of input data. Inclusion of poor data will compound the problems of interpreting ‘natural’ variance, and will probably generate bad predictions. In this work we try to explain how ecotoxicity data obtained in mixtures studies could change de SSD curves for chemical assayed as alone. As first approach we run mixtures of Imazamox-Ammonium on groundwater copepods. SSDs for ammonium considering freshwater Cladocerans, Copepods and Amphipods, EC50 data from single assays and using EC50 data for ammonium in the mixtures were constructed. The binary mixture IMA- NH_4^+ was more toxic when compared their toxicities as single chemicals. The latter was true for both species studied. On the other hand the differences in sensitivity between species of copepod epigean and hypogean, views for ammonium studied separately virtually disappear when compared its LC50 values of both species in the mix. The mixture was found to be synergistic with regard to the action of toxic evaluated separately. Data for E. serrulatus fitted for IA reference modelshowing the lower sum of squared residuals (SS) with respect the deviation patterns. Considering the CA approach data for E. serrulatus not fitted according this, on the contrary a synergistic and dose ratio dependent deviation were evidenced. In this regard the isobologram presented shows a clear synergist effect as a function of dose ratio between ammonium and IMA. In the case of D. belgicus neither CA nor IA reference models fitted at lower SS values. On the contrary a significant synergistic effect was registered when data were subjects to CA model. For the analysis under IA model also was verified a dose ratio effect. SSD obtained without consider EC50 for NH_4^+ in the mixture for both species with IMA underestimate the fraction of potentially affected species.

709 Comparative short-term, chronic and mixture toxicity of chemicals in bee species: The role of toxicokinetic and toxicodynamic traits

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The toxicity of a range of insecticides, other pesticides and contaminants have been assessed in chronic feeding tests with three bee species: Apis mellifera, Bombus terrestris audax and Osmia bicornis. Based on the data derived from these time series exposures, we were able to fit dynamic energy budget toxicity (DEBtox) models. These parameterisations allowed species variations for a range of key toxicokinetic and toxicodynamic parameters for each chemicals to be identified. From fitted models, we were also able to establish the full time course of expected effects on apical endpoints. This analysis allowed us to consider the consequences of life-time exposures for summer and overwintering (worker) bee populations. The magnitude of difference between toxicity statistics calculated from typical short-term bioassay results (e.g. 48 or 96 h feeding studies) and the likely actual sensitivity of life-time exposed populations could be generated for each of the three bee species. Analysis showed that short-term test data may sometime provide an assessment of toxicity that is between 3 and 25 fold lower than expected from life-time exposure. To extend our analysis beyond single chemicals, similar chronic feeding studies were conducted for a range of realistic chemical mixtures. Mixture toxicity modelling approaches, including concentration addition and independent action, and also the mechanistic DEBtox models were used to analyse the nature of joint effect in each species. A number of the tested mixtures showed non-interactive (additive) toxicity. However, examples of both potentiation and antagonism were found. The occurred mainly in cases where there was a clear mechanistic basis for the observed effect, such as detoxification system inhibition of substrate competition. Where synergism or antagonism was found, the magnitude of such effects were generally small. However, in each case they were repeated in each of the three study species provide weight of evidence support for the repeatability of these interactive effects. Overall our analyses suggests that both mixture models, and particularly mechanistic models, such as DEBtox, can be valuable tools for improving risk assessment realism particularly if uncertainties associated with interactive effects are included for priority cases.

710 Ecological Risks of Pharmaceuticals and Personal Care Products in Sediments of an Urban Waterway

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Sediment risk assessments typically evaluate chemicals that represent legacy sources of industrial activities (e.g., metals, pesticides, polychlorinated biphenyls). However, in urban waterways subject to runoff from stormwater and combined sewer overflows (CSOs), additional chemical and biological stressors may need to be considered to more fully evaluate all potential ecological risks in sediments. In this study, ecological risks associated with pharmaceuticals and personal care products (collectively referred to as PPCPs) were investigated in surface sediments of Newtown Creek, an intertidal waterway separating the boroughs of Queens and Brooklyn, New York. This area has an extensive history of industrial activity since the 1800s, and the creek currently receives significant freshwater input from several CSO discharges and stormwater. The objective of this study was to evaluate the quantitative risks from sediment PPCPs relative to the risks of other more typical contaminants of concern. Many of the PPCP analytes proved to be suitable tracers for sewage-related CSO input to sediments. Surface sediment PPCP concentrations correlate with those of a number of human pathogen indicators (bacteria) that are recognized tracers for human fecal pollution. Spatial patterns of PPCPs consistently indicated that CSO-derived sediments deposit not only in close proximity to the major CSOs where coarse-grained sediments are found, but also further downstream in regions characterized by finer-grained sediment. Ecological risks from PPCPs in Newtown Creek sediments are driven by endocrine-disrupting alkylphenols and estrogen hormones. Concentrations of these PPCPs in sediment pore water often exceed aquatic chronic toxicity thresholds by more than a factor of 5. Over-the-counter pharmaceuticals pose relatively little potential risk, rarely exceeding toxicity thresholds. The greatest risks from PPCPs are associated with sediments deposited in tributaries to the creek which receive discharge from the largest CSOs. Additionally, within these CSO tributaries the PPCPs pose unacceptable risk at levels similar to or exceeding those of “traditional” contaminants of concern like PCBs, PAHs, and metals.

711 Ecological Risk Assessment Crossroads between an NJDEP LSRP-led and EPA-Led Cleanup

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Langan was tasked with a implementing a multi-phased ecological risk assessment (ERA) investigation of a wetland and surface water drainage system associated with a former gas production facility located in northwestern New Jersey. Investigation activities were completed to characterize and evaluate the ecological risk associated with site-related contaminants of potential ecological concern (COPEC), including polychlorinated biphenyls (PCBs) and metals. Multiple lines of evidence were used to differentiate and understand the sources and risks of impacts to the wetland and surface water drainage system. During the investigation off-site contributions were identified from an adjacent Superfund. The Superfund site contributions changed a seemingly straight forward ERA into a complicated investigation of a mixture of impacts from the former gas production facility and the neighboring Superfund site. Additionally, a sense of urgency and potential complexity was added to this project as a result of a proposed county roadway development project within the vicinity of both sites. Using the established lines of evidence, Langan was able to identify and quantify the ecological risk associated with site-related sediment impacts and initiate development of a remedial approach to address site-related impacts.

Ecological Effect Models for Assessing the Risks of Pesticides: Ongoing Developments in the US and EU

712 How population models can help to assess the risks of pesticides to threatened and endangered species

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This presentation will demonstrate why and how population models are needed to assess the risks of pesticides to species listed under the US Endangered Species Act (ESA). Despite a long history of population models used in conservation biology and resource management, application of population models for pesticide risk assessments under the ESA has been minimal. We conducted a literature review to explore the availability of appropriate population models and to quantify the extent to which they have been used in listed species risk assessments compared to other applications. In addition, we categorized the reviewed models in terms of their structure, taxonomic coverage, purpose, inputs and outputs, and whether they included density dependence, stochasticity, or risk estimates, or were spatially explicit. Our review covered 403 studies that used population models to inform species conservation and management, however only two of these published studies used population models to assess the risks of pesticides to listed species (both were models of salmon). We conclude that there is an untapped potential to adapt existing models for pesticide risk assessments under the ESA, but also that there are some challenges to do so for listed species. Species-specific life histories and ecology have an important influence on population risk resulting in no direct proportionality between organism- level toxic responses and their population-level impacts. We argue that species- or group-specific population models, in combination with toxicity data for non-listed species, provide an effective approach that can inform both screening-level and more species-specific assessments of risks to threatened or endangered populations and species.

713 Application of a population model for a threatened plant species in herbicide risk assessment

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Extrapolating from organism-level endpoints, as generated from standard pesticide toxicity tests, to populations is an important step in threatened and endangered species risk assessments. Population modeling approaches can be used as tools to estimate potential risk from pesticides to sensitive populations by integrating multiple sub-lethal and lethal effects simultaneously and by accounting for differences in species' life histories. We apply a population model for a threatened herbaceous plant species, Boltonia decurrens, to estimate the potential population-level impacts of different herbicides. We combine conservative in-habitat exposure scenarios with dose-response curves for growth and survival of standard test species, and apply those in the species-specific model. In the model, a yearly herbicide exposure from drift is linked to dose-response curves derived from vegetative vigor tests and affects established plants. Dose-response curves derived from seedling emergence tests are applied to model the effects of exposure from herbicides transported via runoff to emerging seedlings. Exposures are distributed across the simulated habitat applying the RegDISP model for spray drift, and a combination of the Pesticide Root Zone Model (PRZM) and the Vegetative Filter Strip Model (VFSMOD) for runoff. The distributed exposure modeling approach makes it possible to assess potential effects of herbicides on plant populations growing in habitats that border chemical use areas/fields and can be used to assess the effectiveness of mitigation measures such as in-field spray buffer zones. We show that responses of organism-level endpoints are not proportional to modeled population-level effects of pesticides. Specifically, comparison of dose-response curves from standard toxicity test species with the output of the population model demonstrates that the most sensitive organism-level endpoint is not predictive of population-level impacts. In addition, the model results suggest that in-field spray buffer zones can considerably reduce potential effects on populations of B. decurrens growing at the edge of a field. Our case study presents how species-specific population models can be applied in pesticide risk assessment bringing organism-level endpoints, exposure assumptions and species characteristics together in an ecologically relevant context.

714 Population-level risk assessment for an endangered butterfly species exposed to chlorpyrifos

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The United States Environmental Protection Agency, US Fish and Wildlife Service and National Marine Fisheries Service are currently working to develop methods for assessing population-level risks of pesticides to listed species. The draft biological evaluations, released in April 2016, are focused on three organophosphate insecticides, one of which is chlorpyrifos. As these pesticides kill a broad spectrum of insect pests, including several in the order Lepidoptera, there is particular concern for the 34 listed butterfly species. Many of these populations are experiencing significant declines due to dwindling habitat and other stressors. One such species, the Poweshiek skipperling (Oarisma poweshiek) is obligate to remnant prairies, which co-occur with major crops where chlorpyrifos is applied (e.g., corn, wheat, soybeans). This presentation considers methods to assess the percent of habitat and proportion of individuals of this species potentially exposed to chlorpyrifos over a 15 year period of use, and the magnitude of effects at predicted exposure concentrations. At the landscape scale relevant to this population, we examine how exposure may vary by comparing maximum allowable chlorpyrifos use with that predicted when considering factors such as annual variability in crop rotation through different years of land cover data from the US Department of Agriculture's cropland data layer, relevant application timing based on known pest pressure, and historical estimates of percent crop treated with chlorpyrifos in different regions. Contact exposures to larvae or adult butterflies and dietary exposure to larvae consuming grass or adults consuming nectar are considered the dominant routes of concern. Exposure is the result of spray drift transport from adjacent treated fields to prairie habitats inhabited by this listed species. Dose-response curves representing mortality for larvae and adult butterflies are used with spray drift deposition estimates to derive the magnitude of mortality in the exposed portion of the population. This presentation focuses on population-level methods for assessing direct effects of an insecticide, using chlorpyrifos and the Poweshiek Skipperling as an illustration. We will also discuss the applicability of these approaches to Lepidopteran species in general, based on our knowledge of the current status of these listed species and considering species that may be experiencing severe decline.

715 An aquatic food web and ecosystem model for estimating direct and indirect effects of pesticides

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The Comprehensive Aquatic Systems Model (CASM) has been adapted to three models that estimate the direct and indirect toxic effects of pesticides on populations of aquatic plants and consumer organisms representative of Midwestern US lower-order streams, farm ponds, and emergent wetlands. The CASM computes the daily biomass of modeled populations for baseline (no pesticides) conditions and for exposure scenarios defined by daily concentrations of pesticide for 365 days. Baseline population dynamics reflect daily changes in environmental conditions characteristic of the modeled aquatic systems. The CASM provides alternative formulations (e.g., probit, triangular, piece-wise linear) of population-specific exposure-response functions that alter baseline bioenergetics-based growth and mortality parameters as a function of dissolved or bioaccumulated pesticide concentration for each day of exposure. The CASM estimates population, community, and ecosystem-level effects of pesticide exposure based on time-integrated differences between baseline and exposure scenario simulations. The CASM provides a capability to examine the lethal and sublethal, direct and indirect effects (e.g., alterations in within-guild competition, trophic cascades, and nutrient cycling) of time-varying pesticide exposures. The CASM has been developed within a Monte Carlo framework to permit probabilistic risk estimation and to facilitate model sensitivity and uncertainty analysis. The presentation describes the overall modeling approach and presents results for selected pesticides and exposure scenarios in the Midwestern US.

716 An ecotoxicological module for BEEHAVE to link standard laboratory tests to honeybee colony dynamics

P. Thorbek, Syngenta / Environmetal Safety; T. Preuss, Bayer CropScience / Environmental Modelling

The honeybee colony model BEEHAVE was developed by Becher et al. (2014) to assess the effects and interactions of multiple stressors on honeybee colony dynamics, e.g. varroa mites and the viruses they vector, forage shortage and potentially pesticides. Since then it has been evaluated by the European Food Safety Agency (EFSA) who concluded that it could form a sound basis for both setting of protection goals and, with further development, for risk assessment for honeybees. However, EFSA highlighted the absence of a specific ecotox (pesticide) module as a shortcoming. Here we present an ecotox module for BEEHAVE that can use the results from standard tests (e.g. honeybee acute oral, honeybee larval toxicity test) as inputs and predict impacts and, if any, time to recovery for the colony. Small modifications were made to BEEHAVE to allow for co-occurrence of varroa mites and pesticides, the use of dose-response relationships to predict initial effects, exposure via different routes and of different life stages. The BEEHAVE ecotox module will be validated against the results of tunnel (semi-field) studies to assess its ability to predict the initial effects that were observed in the studies.

717 Why Point Estimates of Mortality May Result in Inaccurate Population Modeling and Pesticide Risk Assessment

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Historically, point estimates such as the median lethal concentration (LC50) have been instrumental in assessing risks associated with toxicants to rare or economically important species. In recent years, growing awareness of the shortcomings of this approach has led to an increased focus on analyses using population endpoints and modeling. However, risk assessment of pesticides still relies heavily on point estimates of effect, especially the LC/LD50. However, the LC50 is an estimate, not an absolute number and it has an associated probability and therefore lies within a 95% confidence limit. In this presentation, we will show several examples of matrix population models that have been adjusted with only the LC50 and with the tails of the 95% CL. The results show that population-level outcomes can vary greatly when the tails of the 95%CL are considered ranging from extinction to full recovery. The implications of these results and the need to incorporate variability and uncertainty in point estimates for use in risk assessment will be discussed.

718 Quantifying uncertainty in toxicokinetic-toxicodynamic model predictions

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Toxicokinetic-toxicodynamic (TK/TD) models quantify the expected chemically-induced mortality from any time-variable, environmentally relevant exposure profile, leading to more realistic predictions of individual and population level effects on sensitive species. The flexibility of TK/TD models enables their use for different extrapolation purposes of relevance in risk assessment, for example the evaluation of potential toxic effects from the modelled or measured exposure time series surface water bodies. TK/TD models allow directly relating exposure patterns to expected effects, thereby increasing accuracy and decreasing method-associated uncertainty of risk estimates. In addition, TKTD model predictions provide a means for quantifying uncertainty that is associated to biological variability as captured in the TK/TD model parameters. This study shows how information collected during model parameter estimation can be used to analyse uncertainty of model predictions in a probabilistic way. Model parameters were estimated from observed survival data for a set of four pesticides (carbendazim, cypermethrin, dimethoateand malathion), by maximizing the likelihood estimation. To approximate the joint confidence regions of all parameters, those parameter sets from the optimisation procedure that were not rejected in a likelihood ratio test were selected and subsequently used to generate forward predictions. The minimum and maximum of all predictions at each time point can be interpreted as a 95% uncertainty interval on the model predictions (reflecting parameter uncertainty). Model results for the four selected pesticides indicate that the sample size, the quality of data used for model calibration and the properties of the chemical-species interactions (e.g., the speed of the toxicokinetic and toxicodynamic recovery processes) have a direct impact on the parameter estimates, in particular uncertainties, and hence on the precision of model predictions as reflected by the size of the uncertainty bands of survival dynamics plots of and dose-response curves. Deterministic model predictions using best-fit parameters are in this way augmented with information about uncertainty of model predictions. This supports the evaluation of such model predictions for consideration in the regulatory environmental risk assessment of chemicals.

719 Optimizing experimental designs for calibration of TK-TD models

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The General Unified Threshold model of Survival (GUTS) provides a theoretical framework for analyzing stressor effects on survival over time through consistent model equations based on different assumptions about the stressor quantification, the compensatory processes and the nature of the death process. In ecotoxicology, stressors are toxicants characterized by a dose metric, e.g. the concentration in the medium surrounding an organism or inside the organism, or by the damage quantity they cause. The key GUTS feature is that mortality is estimated when the dose metric exceeds a certain threshold. Several GUTS flavors can be derived according to the assumption underlying the death process: (i) the threshold is distributed within a population, and when exceeded, the individual dies (individual tolerance, IT); (ii) there is one common threshold for all individuals, and when exceeded, the probability to die increases (stochastic death, SD); (iii) a unification of both previous assumptions (GUTS proper). While more realistic, GUTS proper requires the estimation of one additional parameter. Because environmental risk assessment of chemicals depends on robust estimates of GUTS parameters, we investigated parameter identification for GUTS proper, in relation to the experimental design of 'short-term' laboratory bioassays. In practice, standard survival datasets generally do not contain enough information to estimate all parameters of GUTS proper with sufficient precision. This is because a large number of individuals is required to provide strong information on probabilistic events. Hence, based on simulated datasets we identify appropriate experimental designs suitable to estimate all parameters of GUTS proper with the best possible precision. We show that datasets with a high number of animals per treatment allow for parameter estimation of GUTS proper with reasonable accuracy and precision. Moreover, increasing the number of animals or the duration of the experiment substantially reduce the uncertainty around the median value of the threshold. Nevertheless, general statements about optimisation for any chemical, any species, any test duration and/or any exposure concentration profile remain difficult. As take-home message, to the extent possible, we recommend not to use fixed experimental set-up for GUTS analyses, but rather tailor dedicated designs according to the chemical, the species and/or the research/regulatory question at hand.

Recovery of Pelagic Fishery Populations Following Oil Exposures

720 Assessing impacts of crude oil exposure across multiple levels of biological organization in a pelagic marine teleost, the mahi-mahi

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The Deepwater Horizon oil spill in 2010, the largest marine oil spill in US history, resulted in exposure of early life stages (ELS) from spawning pelagic fish species including mahi-mahi (Coryphaena hippurus) over a period of several months. Observations of impaired swimming performance in young adult mahi following only 24 hours of exposure to water accommodated fractions (WAF) of surface slick oil obtained from the Gulf of Mexico during the spill (< 10 µg ∑PAH/l) was paralleled by reduced maximal metabolic rate (MMR), suggesting impaired cardiac function in WAF exposed fish. Subsequent studies demonstrated reduced in situ cardiac output in WAF exposed adult as well as larval mahi-mahi, an effect driven mainly by reductions in stroke volume rather than heart rate. The reduced cardiac output probably explains observations of reduced MMR and swim performance. Isolated cardiomyocytes, when exposed acutely to WAF, show reduced sarcomere shortening and reduced maximal current through L-type Ca^{2+} channels. Furthermore, WAF exposure results in alterations of action potential duration in myocytes, likely explained by observed alterations in inward rectifier and delayed rectifier K^+ currents. The observations of reduced sarcomere shortening and altered ion currents through Ca^{2+} and K^+ channels likely explains, in part, observations of reduced cardiac output. RNAseq experiments on WAF exposed larval mahi displayed altered expression of a number of genes involved in cardiac muscle and Ca^{2+} homeostasis following 24-96 hours of exposure, confirming cardiotoxicity also at the molecular level. Ongoing satellite tagging efforts of adult mahi-mahi aim to test the impacts of WAF exposure on fish in the wild. The expectation is that altered ion currents in isolated myocytes, which leads to reduced contractility and thereby cardiac output and swim performance will result in reduced overall performance expressed as growth, reproduction and survival rates of mahi-mahi in the wild. This research was made possible by a grant from The Gulf of Mexico Research Initiative. Data are publicly available through the Gulf of Mexico Research Initiative Information & Data Cooperative (GRIIDC) at https://data.gulfresearchinitiative.org.

721 Eco-physiological implications of acute embryonic and juvenile oil exposure in marine fish

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Oil exposure has a well catalogued suite of effects on early life stage fishes. These effects are largely caused by the polycyclic aromatic hydrocarbon (PAH) compounds within oil, and include pericardial edema, craniofacial deformities, spinal curvature and mortality. Pericardial edema has been repeatedly demonstrated as the most sensitive measure of PAH exposure in fishes, and ostensibly leads to reduction in cardiorespiratory performance. Similar inhibition is also thought to occur in older life stages by interfering with cardiac excitation-contraction pathways. Cardiorespiratory performance governs the ability of vertebrates to transport oxygen from the environment to the tissues; a process even more limiting in aquatic organisms due to the proportionally low amount of oxygen in water. As such, a comprised cardiorespiratory system can result in a suite of sub-lethal eco-physiological complications for fishes. Here we will discuss the impacts of environmentally relevant (≤ 40 µg l^{-1} $SPAH_{50}$) sub-lethal oil exposure, both embryonic and juvenile life stage exposure, on a variety of ecologically relevant performance measures on a fast growing marine species, the red drum. This will include direct measures of ecological performance such as swimming and aerobic scope, as well as predator-prey and routine foraging dynamics. We will also examine additional measures of ecological performance that manifest through competitive scenarios, including direct dyadic social interactions, group social behavior, individual behavioral syndromes and long-term growth and survival. This research was made possible by a grant from The Gulf of Mexico Research Initiative. Data are publicly available through the Gulf of Mexico Research Initiative Information & Data Cooperative (GRIIDC) at https://data.gulfresearchinitiative.org.

722 Shaken, stirred, neat, or on the rocks: crude oil developmental cardiotoxicity in fish is independent of exposure regimen

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Most laboratory studies on the developmental toxicity of crude oil to fish have been based on scenarios derived from the 1989 Exxon Valdez oil spill. That spill produced a large surface slick that was subjected to high wave energy from storms, followed by the shoreline deposition of mousse on cobble beaches. Methods developed to mimic these habitat conditions included, respectively, high-energy dispersion of oil droplets into seawater with a specialized apparatus (paint stirrer/fan motor) and the now well-known oiled-gravel generator column. Several groups have criticized these exposure methods as too variable or too difficult to control, and advocated instead for standardized methods that avoid mechanical dispersion of droplets into the water column (e.g., the CROSERF method). The subsequent 2010 Deepwater Horizon incident was characterized by the mechanical and chemical dispersion of oil into small droplets under high pressure at the sea floor. In addition, highly weathered material became stranded in nearshore bays and marshes characterized by low surface wave energy. To address the former conditions, we previously developed a simple, easily standardized and reproducible method to mechanically disperse crude oils into seawater using a commercial blender, generating high-energy water accommodated fractions (HEWAFs). Using red drum (Sciaenops ocellatus), a nearshore species of the Gulf of Mexico that produces pelagic embryos and yolk sac larvae, we compared the HEWAF method to low-energy WAFs prepared by the CROSERF method. Using a variety of functional and morphological endpoints for developmental cardiotoxicity, we show the impacts of oil exposure are practically identical for both high- and low-energy methods. In comparison to other Gulf species (e.g., tunas, mahi), red drum embryos are intermediate in sensitivity, showing both relatively severe impacts on heart function and development, as well as secondary abnormal morphology phenotypes. In conjunction with data obtained from other marine and freshwater fish species, and other geologically distinct crude oils, we demonstrate that teleost embryos display a highly consistent and conserved cardiotoxic response to crude oil, irrespective of how it enters the aquatic environment.

723 Fleshing out crude oil cardiotoxicity adverse outcome pathways with transcriptomics in Atlantic haddock embryos

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A major movement to improve risk assessments includes the development and use of adverse outcome pathways (AOPs). AOPs are derived from detailed toxicological cause and effect relationships that span multiple levels of biological organization, ideally from molecular initiating events to ecosystem scale responses. For more than a decade, zebrafish have been the primary experimental platform for mechanistic work on crude oil developmental toxicity in fish. While the zebrafish model has provided the initial cardiotoxicity AOP framework, it is a tropical freshwater species, while the worldÌ_s most valuable fisheries generally spawn in marine and estuarine habitats. Therefore, cross-species extrapolations remain a source of uncertainty for ecological risk assessments. Atlantic haddock (Melanogrammus aeglefinus) provides a number of pertinent features that make it an ideal subject for fully developing crude oil AOPs for marine fish with pelagic early life history stages, including large translucent embryos that provide sufficient mass for chemical analyses, a developmental rate that allows greater resolution between time points, and a sequenced genome. Atlantic haddock thus makes an excellent bridge between zebrafish and other non-model fish with less readily available embryos. The pelagic embryos and larvae of haddock were exposed to Norwegian Sea crude oil using a sophisticated continuous flow system providing controlled delivery of mechanically dispersed oil microdroplets and corresponding water accommodated fraction. We characterized visible cardiac function and morphological phenotypes and obtained corresponding RNASeq data from 6 developmental stages from embryonic exposures, and 5 stages during larval exposure. Cardiac function defects were dose-dependent and included bradycardia and ventricular asystole in embryos and atrioventricular conduction block in larvae. Embryos displayed dose-dependent failure of cardiac looping and reduced ventricular outgrowth. Gene expression data identified changes in key genes encoding specific components regulating cardiac action potentials and excitation-contraction coupling, and identified a clear cause-effect chain linking disruption of intracellular calcium handling to signaling molecules and transcription factors regulating cardiac morphogenesis. These findings thus provide major advances in delineating key molecular events in the developmental cardiotoxicity AOP from crude oil exposure.

724 Time- and oil-dependent genomic and physiological responses to Deepwater Horizon oil in mahi-mahi (Coryphaena hippurus) embryos

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The Deepwater Horizon (DWH) oil spill contaminated the spawning habitats for numerous commercially and ecologically important fishes. Exposure to the water accommodated fraction (WAF) of oil from the spill has been shown to cause cardiac toxicity during early developmental stages across fishes. To better understand the molecular initiation events and explore new pathways responsible for toxicity, RNA sequencing was performed in conjunction with physiological and morphological assessments to analyze the time-course (24, 48 and 96 hour post fertilization (hpf)) of transcriptional and developmental responses in embryos/larvae of mahi-mahi exposed to WAF of weathered (slick) and source DWH oils. Slick oil exposure induced more pronounced changes in gene expression over time than did source oil exposure. Predominant transcriptomic responses included alteration of EIF2 signaling, steroid biosynthesis, ribosome biogenesis and activation of the cytochrome P450 pathway. At 96 hpf, slick oil exposure resulted in significant perturbations in in eye development and peripheral nervous system, suggesting novel targets in addition to the heart may be involved in the developmental toxicity of DHW oil. Comparisons of changes of cardiac genes with phenotypic responses were consistent with reduced heart rate and increased pericardial edema in larvae exposed to slick oil but not source oil.

725 The effects of photoproducts generated from irradiated crude oil exposures on pelagic and estuarine fish species

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Photo-induced toxicity occurs when ultraviolet (UV) radiation excites photoactive polycyclic aromatic hydrocarbons (PAHs) to produce reactive oxygen species (ROS) and modified PAH photoproducts, resulting in increased mortality. Several studies following the Deepwater Horizon (DWH) oil spill have shown that co-exposure of UV radiation and crude oil to aquatic organisms significantly enhances toxicity. However, the effects of photoproducts generated during a crude oil spill have not been studied on pelagic or estuarine fish species. In this study, high energy water accommodated fractions (HEWAFs) of Australian Northwest Shelf (NWS), DWH oil from the surface (OFS), and DWH source oil b (SOB) were diluted and irradiated by ambient UV sunlight for 4-6 h to generate modified PAH photo-products. Two pelagic species, mahi mahi (Coryphaena hippurus) and yellowtail kingfish (Seriola lalandi), and two estuarine species, black bream (Acanthopagrus butcheri) and red drum (Sciaenops ocellatus) were exposed to dilutions of the irradiated HEWAFs. The effects on survival and cardiotoxicity were quantified in all fish species. Preliminary chemical analysis of the irradiated HEWAFs indicated that the UV radiation generated modified PAH photoproducts that were associated with cardiotoxicity in these studies. Further, the irradiated HEWAFs enhanced toxicity as high as six fold in the black bream and mahi mahi, compared to the non-irradiated counterpart. This research provides evidence that photoproduct formation during UV exposure contributes to the photo-enhanced toxicity of crude oil to pelagic and estuarine fish species. This research was made possible in part by a grant from The Gulf of Mexico Research Initiative, and in part by CSIRO Oceans and Atmosphere. Data are publicly available through the Gulf of Mexico Research Initiative Information & Data Cooperative (GRIIDC) at https://data.gulfresearchinitiative.org.

726 Phototoxic Target Lipid Model (PTLM) of Single PAHs and Mixtures

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A model of PAH acute phototoxicity (PTLM) will be presented that is based on the target lipid model (TLM) of PAHs and other narcotic chemicals. It can predict either the LC50 concentration measured at a fixed duration of irradiance exposure, or the LT50, the time required to achieve 50% mortality at a fixed concentration. The inputs to the model are the molar adsorption spectra of the PAH of concern, the spectra of the light irradiance, and the duration of exposure to light. These are used to compute the energy absorbed by the PAH, P_{abs}, with units moles of photons absorbed per mole of PAH. The only species dependent parameter in the model is the critical target lipid body burden (CTLBB), which is obtained from the TLM. It is the organism lipid normalized body burden that produces 50% mortality. They apply to any narcotic chemical and are currently available for 47 aquatic animals and five algal species. The model has two parameters that are fit by calibration to a data set compiled from the literature comprising 20 individual PAHs and 15 test species. They are (1) the ratio of organism critical body burden for reactive phototoxic species, formed by the energy absorbed by the organism's PAH body burden, to the CTLBB; and (2) an exponent, a, that quantifies the nonlinear response of mortality to energy absorbed $(P_{abs})^a$. The model is validated by application to 12 alkylated PAHs and other phototoxic chemicals in oil. It is applied to mixtures of PAHs by assuming additivity of the toxic units computed for each of the PAHs in the mixture. The results of the calibration, its application to mixtures of PAHs, and to water accommodated fractions from various neat and weathered petroleum samples, will be presented.

727 DWH Oil Spill: Field evaluation of biological responses in Gulf sturgeon and laboratory exposure of shovelnose sturgeon to artificially-weathered oil

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The Deepwater Horizon (DWH) oil spill began in April 2010 and ultimately released 3.19 million barrels of oil into the Gulf of Mexico (Gulf). Natural resources impacted by this disaster included numerous species of fish and wildlife of economic and ecological value, including the threatened Gulf sturgeon (Acipenser oxyrinchus desotoi). Gulf sturgeon are anadromous and were in freshwater tributaries spawning while the spill occurred and they returned to the Gulf in the fall. The objective of our study was to evaluate the potential influence of oil exposure on the health of Gulf sturgeon from all northern Gulf populations. Over 500 Gulf sturgeon were geospatially tracked by acoustic telemetry to evaluate extent and duration of oil exposure. Molecular and physiological indicators of fish health including whole blood DNA fragmentation, DNA repair protein, cell cycle, histochemistry, and gene expression profiles were evaluated in Gulf sturgeon from three genotypic populations. Additionally, shovelnose sturgeon (Scaphirhynchus platorynchus), were exposed to artificially weathered DWH oil through a high energy water accommodated fraction (HEWAF) at an ecologically-relevant concentration of 0.005 – 0.020% for 30d. Organ weights and hepatic CYP1A induction were measured in lab exposed sturgeon in addition to the blood-based health indicators. Gene expression profiles indicated alterations in pathways associated with immune system function, blood flow, neutrophil processes, T and B cell processes, wound healing, and DNA replication in lab exposed sturgeon, consistent with organ changes in the spleen, liver, and CYP1A induction. Whole blood DNA fragmentation and repair in Gulf sturgeon populations were not different prior to entry into the Gulf in 2010, consistent with predicted exposure to DWH oil when returning to freshwater tributaries, and exhibiting more DNA repair capability in the second year. Pathway analysis of gene expression in Gulf sturgeon indicated alterations in immune processes as well as DNA repair. Indications of oil-induced stress were evident in both the laboratory-exposed shovelnose sturgeon and field populations of Gulf sturgeon.

Alternative Approaches to Complex Environmental Challenges – Part 2

728 Integrated Management of Water Resources using Fractal Analysis

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Kumbhmela, the most popular festival in India where Hindu devotees take the bath in the waters of Sangam, the confluence of the holy rivers Ganges, Yamuna and (mythical) Saraswati, is famous worldwide for its sacredness. During such festival, a lot of devotees across the world take the holy dip in the water leading to serious water pollution decreasing aesthetic value of rivers. An attempt has been made in order to determine the spatial distribution of river water quality parameters and to identify places with the best quality for the different purpose within the study area based on (1) use of Geographical Information System and (2) Water Quality Index (WQI) calculation, and (3) fractal analysis. Using GIS contouring methods with ArcGIS 10.0, spatial distribution maps of pH, turbidity, TDS, BOD, DO and total coliform were analysed. The spatial distribution map shows that a majority of the river water samples falls in the very poor category. WQI was used to assess the suitability of river water from the study area for human consumption. From the WQI assessment, over 90% of the water samples fall within the ''Poor'' and ''Very poor'' categories, suggesting that river water from these unsuitable for drinking purposes. Whereas fractal analysis was applied by using year-long water quality data to predict the water quality data for subsequent festive years. A correlation was drawn with the number of people attending such events with that of the water quality. This tool can be applied to any river for the prediction of water quality across the globe during festive seasons when river water is being widely exploited.

729 FAA's Environmental Toxicology and Emissions Assessment of Alternative Piston Aviation Fuels

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The Federal Aviation Administration (FAA) shares the Environmental Protection Agency's (EPA) concerns about lead emissions from small aircraft. Owners and operators of more than 200,000 piston-engine aircraft operating in the United States rely on leaded aviation gasoline (avgas) to power their aircraft. Because of lead toxicity and environmental persistence, the FAA and EPA and industry are partnering to remove lead from avgas. Avgas emissions have become the largest contributor to the relatively low levels of lead emissions produced in the U.S. As a result, the Piston Aviation Fuel Initiative (PAFI) was established at the request of a broad cross section of the aviation and petroleum industries and consumer representatives to develop a path forward for the identification, evaluation and deployment of the most promising unleaded replacements for avgas. The mission of PAFI is to evaluate candidate unleaded replacement fuels and identify those fuels best able to technically satisfy the needs of the existing aircraft fleet while also considering the production, distribution, cost, availability, environmental and health impacts of those fuels. The FAA selected four unleaded fuels to undergo Phase I testing at the agency's William J. Hughes Technical Center. Phase I included environmental toxicology, emissions, engine and rig performance, and materials compatibility. This presentation focuses on the assessment framework for environmental toxicology and engine exhaust emissions. A risk-based approach was followed based largely on published toxicological data and physical/chemical properties of fuel components, comparing potential human health and ecological risks of the unleaded fuels to those of avgas. Engine exhaust emissions tests included analysis of total hydrocarbons, carbon monoxide, carbon dioxide, nitrogen oxides (NO, NO_2, and NO_x), methane, sulfur dioxide, acetaldehyde, formaldehyde, and particulate matter. Based on Phase I test results, two fuels were down selected to enter more comprehensive Phase II testing, with the eventual replacement of leaded avgas in 2018.

730 Sediment homogenization and aging alters the toxicity of metal-amended sediments

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Metal-amended sediment is commonly used in laboratory studies to establish and verify sediment quality guidelines; however, the similarity between lab-prepared sediment and naturally contaminated sediment may be limited by experimental conditions (e.g., water renewal, pH buffering, equilibration time). A series of laboratory experiments explored how the existence of vertical heterogeneity in redox (i.e., oxic surface sediment overlying anoxic sediment) could modify sediment toxicity to Hyalella azteca. Geochemically distinct sediments were amended with Ni, Cu, and Zn in separate experiments, and toxicity thresholds were calculated using standard 28-day H. azteca bioassays. Metal amended sediment was then added to a flow-through flume to age for >100 days. During aging of the Cu and Ni amended sediments, caged H. azteca were placed on the sediment surface to monitor toxicity (7-day growth). During aging, metal concentrations were stable, but aged sediment was less toxic to H. azteca than freshly amended sediment. Spatially resolved sediment geochemistry revealed that the change in toxicity during aging was related to changes in metal speciation during the oxidation of surface sediment. Aged sediment was removed from the flume, homogenized, and placed in beakers for standard 28-d H. azteca bioassays. Surprisingly, the toxicity of aged sediment was more similar to results observed during initial beaker tests when compared to toxicity of intact aged sediment. Most notably, one sediment from each of the Cu and Ni tests elicited no toxicity at all concentrations tested following >100 days of aging flume, but when homogenized and placed in beakers the toxicity returned. Homogenization of sediments prior to 28-d bioassays likely eliminated redox and associated geochemical gradients in surface sediments that developed during aging, thus counteracting any effects of aging observed in mesocosm experiments with intact sediments. This study demonstrates the importance of properly designed sediment preparation methods for accurate concentration–response thresholds, but aging prior to standard 28-d bioassays does not seem to alter sediment toxicity when homogenization is required. However, we advocate more study of intact sediment cores to elucidate how homogenization of sediments prior to testing may modify metal geochemistry and toxicity to epibenthic invertebrates.

731 Extending a Toxic-Unit Model from Insect Prey to Bats: Pitfalls and Potential

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Metal contamination in freshwater ecosystems is a widespread consequence of mining activities, but effects on nearby riparian insectivores are poorly understood. In this study we attempted to extend a toxic-unit model of mixed metal bioavailability to predict bat habitat use. We hypothesized that more contaminated stream reaches (>3,000 m) in the Colorado Mineral Belt would have lower proximate bat activity due to reduced densities of adult aquatic emergent insect prey. From July to October 2014, we sampled twelve sites for bat activity, stream heavy metal concentrations, and aquatic emergent insect densities. There was no relationship between total bat activity and aqueous metal concentration, despite a confirmed negative correlation between metal concentration and aquatic emergent insect biomass. However, there was a trend toward streams with lower metal concentrations having more bat prey capture attempts (as measured by feeding buzzes) than streams with higher metal concentrations. These data suggest that although bats are passing over streams of all contamination levels at a similar rate, they may be encountering prey more often over cleaner streams. While this study highlights difficulties in determining sub-lethal effects on bats, the model may yet be a useful tool for assessing risk in higher-productivity habitats.

732 Assessing and Managing Multiple Risks in a Changing World – the Roskilde Recommendations

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Roskilde University hosted a November 2015 workshop on "Environmental Risk – Assessing and Managing Multiple Risks in a Changing World". Thirty attendees from 9 countries developed consensus recommendations regarding: implementation of a common currency (ecosystem services) for holistic environmental risk assessment and management; improvements to risk assessment and management in a complex, human-modified, and changing world; appropriate development of protection goals in a 2-stage process involving development of a) universal, and b) site-, region-, or problem-specific protection goals; addressing societal issues; risk management information needs; conducting risk assessment of risk management; and development of adaptive and flexible regulatory systems. We encourage both cross- and inter-disciplinary approaches to address 10 recommendations: 1) adopt ecosystem services as a common currency for risk assessment and management; 2) consider cumulative stressors (chemical and non-chemical) and determine which dominate to best manage and restore ecosystem services; 3) fully integrate risk managers and communities of interest into the risk assessment process; 4) fully integrate risk assessors and communities of interest into the risk management process; 5) consider socio-economics and increase transparency in both risk assessment and risk management; 6) recognize the ethical rights of humans and ecosystems to an adequate level of protection; 7) determine relevant reference conditions and the proper ecological context for assessments in human-modified systems; 8) assess risks and benefits to humans and ecosystems, and consider unanticipated consequences of management actions; 9) avoid excessive conservatism or possible under-protection resulting from sole reliance on binary, numerical benchmarks; and 10) develop adaptive risk management and regulatory goals based on ranges of uncertainty.

733 Viability of Precision-cut Organotypic Cultures of Marine Mammal Skin: Applicability to in Vitro Toxicology

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Organotypic culture refers to the culture of precision-cut organ slices. This in vitro methodology is amenable to many downstream applications including pharmacological and toxicological studies. Benefits of organotypic culture include the preservation and culture of the entire tissue structure, as opposed to cell culture where a single cell type is cultured. While this methodology is well established in pharmacology and mammalian toxicology studies and validated as closely modeling in vivo processes, it is relatively novel with regards to marine mammal toxicology. In vivo toxicological studies are precluded legally and ethically in marine mammals, thus in vitro studies are needed to understand the mechanisms and processes these animals undergo upon exposure to contaminants. One matrix that can be used for such in vitro studies is the skin and blubber matrix. While a few studies of this nature have been conducted with this matrix, they utilized manually cut organ slices as opposed to the precision-cut methodology reported here. To validate this methodology, we tested the viability of precision-cut bottlenose dolphin (Tursiops truncatus) skin and blubber sustained in culture after 24, 48, and 72h of incubation. We examined three endpoints to assess viability after each incubation time point: (1) cell growth from the cultured organ slices, (2) internal potassium (K^+) concentrations in the cultured organ slices as measured by a flame atomic absorption spectrometer, and (3) lactate dehydrogenase (LDH) activity in media collected from the cultures. Immediately following each time point, 20 mg of cultured tissue was saved for internal K^+ analysis with the remainder of the tissue used to create cell culture explants. Media from each culture was saved for LDH analysis. Fibroblast cell cultures were successfully grown from all organotypic cultures which demonstrated viability of this tissue type sustained in culture up to 72h. Internal K^+ analyses are underway to establish a threshold of viability with this type of analyses as with LDH analysis of the culture media. This data indicate organotypic culture of these precision-cut tissues as a valid methodology, opening the avenue of research to conduct non-lethal, minimally invasive toxicological studies in protected marine mammals. Analyses of bottlenose dolphin skin and blubber biopsies exposed to common marine contaminants are underway.

734 Using blubber to examine steroid hormone homeostasis in bottlenose dolphins exposed to dichlorodiphenyltrichloroethane (DDT)

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Dichlorodiphenyltrichloroethane (DDT) and its metabolites (ΣDDT) are known to impact steroid hormone homeostasis in mammals, but there has been minimal investigation in this area in cetaceans, likely due to the legal and logistical factors that limit use of marine mammals for toxicological studies. The purpose of this study is to examine the impacts of ΣDDT exposure on steroid hormone homeostasis in the bottlenose dolphin (Tursiops truncatus) using alternative analytical methods to overcome these limitations. Blood is the most commonly used tissue for endocrine status assessments, but collection of blood from wild cetaceans is stressful for the animal and logistically difficult for researchers. Conversely, blubber biopsies can be collected remotely by non-lethal projectile and steroid hormones have previously been measured in cetacean blubber, but the relevance of blubber hormone concentrations to the wider endocrine system is poorly understood. In order to contextualize blubber steroid hormone concentrations, we will build a generalized linear model for the calculation of circulating steroid hormone concentrations from blubber hormone measurements in T. truncatus. A liquid chromatography-tandem mass spectrometry (LC-MS/MS) assay will be used to simultaneously assay 17 steroid hormones in matched blood and blubber samples collected from a reference population of T. truncatus. To build the model, these measurements will be incorporated with demographic (sex, age, pregnancy status) and sampling (season, time to capture, and time to sample collection) variables that are known to impact steroid hormone homeostasis. We will use this model and blubber biopsies to assess endocrine status in bottlenose dolphins localized to a site with high environmental concentrations of DDT. Blubber organic contaminant burden will be analyzed by GC-MS. We hypothesize that ΣDDT burden will be negatively associated with steroid hormone concentrations in blubber (measured) and blood (model-predicted). This study will advance our understanding of the effects of DDT exposure on endocrine status in T. truncatus, and could facilitate use of blubber for future studies in other cetaceans.

735 Estimates of Community Exposure and Health Risk to Sulfur Dioxide from Power Plant Emissions Using Short-Term Ambient Air Monitoring

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To estimate plausible health effects associated with peak SO_2 levels from three coal-fired power plants in the Baltimore Maryland area, air monitoring was conducted between June and September 2013. Historically, the summer months are periods when emissions are highest. Monitoring included a 5-d mobile and a subsequent 61-d stationary monitoring study. In the stationary monitoring study, equipment was set up at four sites where models predicted and mobile monitoring data measured the highest average concentrations of SO_2. Continuous monitors recorded ambient concentrations each minute. the 1-min data were used to calculate 5-min and 1-h moving averages for comparison with concentrations from clinical studies that elicit lung function decrement and respiratory symptoms among asthmatics. Maximum daily 5-min moving average concentrations from the mobile monitoring study ranged from 70 to 84 ppb(183-220 ug/m3) and maximum daily 1-h moving average concentrations from the mobile monitoring study ranged from 15 to 24 ppb (39-63 ug/m3). Maximum 5-min moving average concentrations from stationary monitoring ranged from 39 to 229 ppb (102-600 ug/m3), and maximum daily 1-h average concentrations ranged from 15-134 ppb (40-351 ug/m3). Estimated exposure concentrations measured in the vicinity of monitors were below the lowest levels that have demonstrated respiratory symptoms in human clinical studies for healthy exercising asthmatics. Based on 5-min and 1-h monitoring, the exposure levels to SO2 in the vicinity of the C.P. Crane, Brandon Shores and H.A. Wagner power plants were not likely to elicit respiratory symptoms in healthy asthmatics. Implications: Mobile and stationary air monitoring for SO_2 were conducted to quantify short-term exposure risk, to the surrounding community, from peak emissions of three coal-fired power plants in the Baltimore area. Concentrations were typically low, with only a few 5-min averages higher than levels indicated during clinical trials to induce changes in lung capacity for healthy asthmatics engaged in exercise outdoors.

"One Health": Opportunities for SETAC Leadership in Integrating Environmental, Human and Animal Health

736 History and Evolution of One Health: Integrating Human, Animal and Environmental Health

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One Health is an evolving, interdisciplinary way of approaching complex health issues by recognizing the interconnectedness of human health, animal health and the environment. It integrates human, veterinary, wildlife, and environmental health disciplines at multiple levels to face increasing challenges to health, nutrition, security, and economic growth worldwide. One Health seeks to increase communication and collaboration across disciplines in order to promote, improve, and defend the health of all species on the planet. One Health is a framework concept needed to encourage and expand transdisciplinary collaborations in research, practice, policy, and communications for the benefit of people, plants, animals, and ecosystems. Although the term "One Health" is fairly new (~2004), the concept has a long history, both nationally and globally. From ancient Greece and Rome through the Renaissance and into the current era, taxonomic linkages, similarities, and differences among humans and other animals have formed the heart of comparative medicine. In recent years, through the support of key individuals and vital events, the One Health concept has gained explicit recognition. It is increasingly common to see One Health included by name in inter-institutional research partnerships, conferences, communications, and organizational frameworks–particularly those championed by the human health and veterinary medical communities. To date, however, environmental quality contributions to health and wellbeing remain the least developed components of recent collaborations organized under the One Health concept. The One Health approach represents a great opportunity to toxicologists and chemists to reach out for new collaborations by offering their environmental quality expertise to partnerships focused on solutions that work for humans, plants, animals, and the environment.

737 Continuint the One Health Dialogue in SETAC

L. Kapustka, LK Consultancy

SETAC engagement of One Health began at the Salt Lake City SETAC Meeting. There is a tension at the heart of this concept that divides along lines of reductionism and holistic, systems approaches. Instead of either or, these complementary ways understand complex environmental issues that exist in ecological-sociological landscapes. Quantifying substances in complex media has advanced to amazing degrees of precision and accuracy. Despite the ability to analyze situations in great detail, important determinants of environmental conditions including health of humans and ecological entities remain elusive. While we learn more and more about a situation, we miss the connections that really matter. Humans seem predisposed to become specialists and become increasingly isolated from things going on around them – the siloization phenomenon. John Maynard Keynes observed "It is better to be roughly right, than precisely wrong." Ecological-sociological landscapes are regulated by nested feedback loops that defy simple linear causal explanations. There is awareness that instead of a single determinant of health, multiple factors interact in unexpected ways. Poverty and the stresses that come with it make humans more susceptible to illness. Changes in climate in Eastern Africa is one of many contributing factors to the spread of malaria. Similarly, multiple stressors to plants and wildlife, including fragmentation of landscapes can make insults from chemical pollutants more potent. One Health arose from veterinary sciences with the recognition that some diseases are shared by livestock and humans and that livestock and wildlife can give early warning signals of impending problems for humans. The Center for Disease Control and the World Health Organization have adopted One Health programs promoting collaboration among physicians, veterinarians, and ecologists. Combatting the Zika virus will require reliance on the tenets of One Health to be successful. Management of environmental contamination has been dominated by reductionist approaches. Establishing effective policy and programs to deal with the contamination has proven challenging, even before consideration of changing climate conditions. The complexity of futures scenarios demands a holistic systematic approach. To do this, there will be a need for multi- and transdisciplinary collaboration that should be embraced by SETAC. In this overview, I will highlight opportunities and challenges available to SETACers.

738 Multilateral Environmental Agreements on Chemicals and Waste: How can we improve the link between human and ecosystem health?

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There are five key Multilateral Environmental Agreements (MEAs), and a global policy framework (Strategic Approach to International Chemicals Management (SAICM) which aim to reduce the toxic chemical load to our environment. However, of the more than 103 million chemicals registered by the Chemicals Abstracts Service to date, the chemicals listed in the annexes of the MEAs, indicates that only about 130 chemicals are actually covered, with a focus on a limited number of industrial chemicals, persistent organic pollutants ozone depleting substances, a range of non-POPs pesticides, mercury, and some chemical-related wastes. Monitoring data for the last decade indicates that there have been successes using the current a posteriori approach of the MEAs, which have led to reductions in the risk derived from persistent chemicals. The current SAICM (2006) has also contributed by raising concern on chemicals of emerging concern such as nanomaterials, endocrine disruptors etc. But does the current approach respond to the need of sustainable development goals?. Taking a global solution perspective, the aim is to enhance sustainable chemicals management practices throughout the chemical life cycle, minimizing the negative impacts to human health and ecosystems. In the different MEAs the inclusion of human and ecosystem health concepts have been differently applied. For instance, in the POPs Review Committee of the Stockholm Convention,in the listing of new chemicals added to the Convention, there are considerations on human health and ecosystem impacts. The consideration of these elements are based on (eco) toxicological thresholds and a risk profile is elaborated with all the available scientific information. Another example, is the mercury platform work that STAP is developing together SETAC to provide a model of interaction between scientists, and any data owners and data users, capable of contributing to knowledge on global mercury emissions, and impacts on human health and ecosystems. Starting with a pilot of fish mercury data, the intent in practice is to build a body of evidence to assess pollution levels, to improve understanding of fate and transport of mercury in the environment, how communities may be affected, and elaborate the best specific control measures. In the face of a growing list of chemical formulations, we need to increase integrated evidence for the global governance of chemicals, and the One Health concept may be instrumental in that direction.

739 Benefits of a Safer Chemical Ingredient List

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Safer products minimize risk through the selection of the least hazardous ingredients for a particular functional use and product type. The USEPA Safer Choice program has labeled more than 2,000 products, indicating to consumers and other purchasers that a product meets EPA's rigorous Safer Choice Standard. EPA evaluates intentionally added ingredients and residuals against published safer chemical criteria. The Safer Choice Safer Chemical Ingredient List (SCIL) assists product manufacturers in their search for safer chemicals. SCIL currently includes over 750 chemicals that have met the program's criteria, including: surfactants, solvents, preservatives and chelants. Providing transparency from 15 years of identification and assessment of formulated wet chemistry products, SCIL offers product formulators, retailers, researchers, advocates and activists insights on and means to achieve informed substitution and advance green chemistry principles. Chemicals are distinguished using simple color codes that quickly communicate toxicological information for a diverse audience. Criteria for listing were developed in multi-stakeholder public processes and are available to be used for any application. Unlike restricted substance lists and regulatory lists, SCIL empowers formulators to lead in safer chemistry (and avoid tomorrow's "chemical of the week") and suppliers to highlight their green chemistry successes. Even with the successes highlighted in this presentation, additional work on developing safer chemicals needs to be done; for example, several categories-including preservatives and colorants-have limited numbers of chemicals that pass all hazard criteria. Where substances are initially reviewed one at a time or in the context of a small category, the growing SCIL will allow for the re-review of large categories of substances, and improvement in criteria for defining 'safer' chemicals. Part of the EPA strategy for this work is to engage external audiences and expand the list. As the number of restricted substances, or chemicals with negative public attention increase, formulators and the public need a resource to define chemicals unlikely to be restricted. SCIL serves as a guide for safer chemistry grounded in science and 'one health'.

740 One Health: An Organizing Framework for Global Engagement on Coupled Human-Ecological Training and Research?

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SETAC launched the Global Horizon Scanning and Prioritization Project to identify geographically specific research needs aimed at advancing sustainable environmental quality. Priority research questions were solicited from SETAC members and other environmental professionals within the five SETAC geographical units (GU), then synthesized by teams of academic, business and government representatives to develop lists of the top research questions that, if answered, would substantially advance our understanding of how a range of environmental stressors (chemical, physical, biological) impact environmental quality in different geographic regions. During 2015, workshops were held for Africa (in Langebaan, South Africa), Australasia (in Nelson, New Zealand), Latin America (in Buenos Aires, Argentina) and SETAC North America (in Salt Lake City, USA). An Asia Pacific workshop is planned during the Singapore meeting in September. Whereas several consistent patterns of research needs are emerging among GUs, coupled human health and ecological consequences of stressors and research and training capacity emerge from GUs with a number of developing countries. Such observations are critical because global population concentration is increasingly occurring in megacities of countries in transition, where landscape modification and access to diverse chemicals, including consumer products, pesticides and medicines, are increasing more rapidly than waste management infrastructure and other public health intervention strategies are implemented. SETAC's noble mission, multidisciplinary and tripartite membership, and global organization structure is uniquely positioned to contribute to transdisciplinary One Health teams. Timely opportunities, including collaboration with environmental public health professionals and organizations, will be presented as potential opportunities for SETAC members to develop training and collaborative research frameworks across GUs.

741 Assessing Ecosystem and Human Health Effects from Natural and Man-Made Disasters

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Since 1990, natural disasters have globally impacted about 217 million people each year. Recent evidence suggests a substantial increase in such disasters since 1989, increasing from 50- >100 annually from 1950-1970's followed by a rapid to ~275-350/year during 2000-2009. Coupled with this have been the concomitant interactions of increasing urbanization, deforestation, environmental degradation, and climate change. This recent history of disasters in the U.S. underscores a trend of increasing frequency and level of economic damages and effects on ecosystem and human health. In 2005, Hurricane Katrina caused > $85 billion in disaster damages and 1,833 deaths; in 2010 the Deepwater Horizon oil spill was directly responsible for 11 deaths and the release of some 5 million barrels of oil to the Gulf of Mexico ecosystem and in 2012, in there were 11 weather disasters in the U.S. that resulted in 377 deaths and >$110 billion in damages, including Hurricane Sandy which accounted for >$70billion in damages. Most disaster response efforts focus on immediate and near-term injury and exposures (e.g., to infectious disease, toxic substances, or toxins), with relatively little attention to long-term effects on ecosystem and human health. Conceptual models have been widely utilized in many fields of scientific research to organize and visualize information so as to reveal associations, connections, and sometimes even cause-and-effect relationships among elements in complex environments, including ecosystems. These tools may be additionally employed to increase understanding, develop testable hypotheses, and identify appropriate and effective environmental management steps to minimize risks. Despite their long and widespread use, there has been relatively little prior effort to develop conceptual models that relate disaster- or other stressor-caused environmental damage to ecosystem and human health and well-being. Data will be presented on a conceptual model that evaluated ecosystem and human health effects both during and after natural (hurricanes) and man-made (oil spills) disasters. This model provides a framework to improve and better connect both pre-disaster preparation and post-disaster responses in ways that lead to increased understanding of significantly important associations between environmental condition and human health and well-being by assessing both direct and indirect health effects associated with stress directly caused by disasters.

742 Plasma chemistry & persistent organic pollutants in loggerhead sea turtles with & without fibropapillomatosis from Florida Bay & Indian River Lagoon

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Florida Bay and the Indian River Lagoon (IRL) are semi-enclosed coastal ecosystems that have suffered the consequences of anthropogenic alterations. Development and agriculture in Florida have increased runoff of chemical pollutants in both bays, and freshwater diversion has resulted in annual events of hypersalinity in Florida Bay. Some of the sea turtles inhabiting these bays exhibit the tumor-forming disease, fibropapillomatosis (FP). We evaluated plasma chemistry panels and persistent organic pollutants (POPs) in a total of 37 juvenile loggerhead sea turtles (Caretta caretta) at these sites. None of the turtles had moderate or severe FP tumors; only low severity FP was observed in 18 % and 20 % of the sampled Florida Bay and IRL turtles, respectively. POP concentrations did not differ between tumored and non-tumored turtles and were lower in the Florida turtles than loggerheads foraging farther north where FP is rare or absent. These findings support recent conclusions that POPs are not a primary co-factor for the initiation of FP. Florida Bay turtles had significantly higher plasma sodium, chloride, calculated osmolality, and aspartate aminotransferase (AST), and lower albumin, potassium, and POP concentrations, than the IRL turtles. Compared to reference ranges we developed from a meta-analysis of loggerhead plasma chemistry data, Florida Bay turtles, which were sampled over only one week in June 2009, had mildly elevated plasma sodium, chloride, and AST. Using clinical judgement and results from the meta-analysis, we developed criteria for health conditions and categorized 82 % of the sampled Florida Bay turtles with osmoregulatory imbalance, 68 % with AST exceeding those indicative of tissue damage, and 23 % with possible renal insufficiency. IRL turtles were affected to a lesser degree (40 %, 7 %, and 0 %, respectively). We speculate that hypersaline conditions (>40 practical salinity units) that occurred approximately six weeks prior to the sampling event in Florida Bay may have caused the osmoregulatory imbalance, and may make the turtles more susceptible to other health stressors. While loggerhead turtles in the IRL and Florida Bay are exposed to POPs, the toxicological risks appear to be lower relative to other environmental stressors, such as hypersalinity in Florida Bay. Future research should assess the osmoregulation and health of marine organisms across seasons and years in relation to salinity in Florida Bay.

743 One Health Approach Towards Addressing Pollution at the Pole – Integrating Evidence Across Inuit, Wildlife, and Ecosystems

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Many are surprised to learn that Inuit populations, Arctic wildlife, and northern ecosystems are amongst the most contaminated groups worldwide. Here I will outline how One Health principles underlie key aspects of the pollution at the pole discourse. First, traditional belief systems of Inuit are grounded in the notion that everything is connected. Contamination of ecosystems, wildlife, and people are inter-related, and together this has profound and well-documented impacts on socio-cultural systems, economic well being, and total health. Second, Arctic contamination has been of societal concern and scientific interest since the early 1970s, and have been the focus of a number of initiatives such as the Northern Contaminants Program (NCP) initiated in 1991 by the Canadian government and the Arctic Monitoring and Assessment Programme (AMAP), a circumpolar multi-country program established to assess the state of multiple compartments in the Arctic ecosystem. These programs have followed One Health principles, and enabled detailed investigations into the source, fate, and transport of contaminants into the Arctic ecosystem, as well as human health and wildlife risk assessments. Finally, international agreements, including the Stockholm Convention and the Minamata Convention, put important value on evidence from humans, wildlife, and ecosystems, especially as they pertain to the Arctic. Taken together, One Health principles are omnipresent in the global discourse of contamination at the pole, and here I will highlight key examples that emphasize the importance of such an approach in helping translate knowledge into action.

PAHs in the Real World: Sources, Sinks, Bioavailability and Toxicity

744 Assessing PAHs in the Real World

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For the past 4 decades, broad spectrum chemical analysis of environmental samples has typically included the “EPA 16” individual polycyclic aromatic hydrocarbons (PAHs). The data remain useful for such purposes as geographic or historical comparisons, preliminary investigations of legacy sites, or initial forensic evaluations of undifferentiated petrogenic versus pyrogenic sources. PAHs occur in the environment as complex mixtures, however, rather than as individual chemicals, and concentrations of a limited list of individual PAHs is insufficient either to make use of sophisticated methods of PAH evaluation or to advance understanding risks associated with PAHs in the environment. Assessing environmental mixtures of PAHs involves recognition of complicated processes that can vary from location to location and differ in different media (air, soil, sediment, biota). One approach to unraveling the complexity is to analyze for a longer list of individual PAHs and including heterocyclic compounds or alkylated or substituted PAHs. Adding to the list of analytes furthers precautionary risk assessments based on assumptions of additive toxicity. Expanded lists of individual analytes, however, does not alter the actual toxicity of PAH-containing materials. Indeed, including more compounds when using an additive approach may exacerbate overestimation of risks. Use of a longer list of compounds may, in some cases, add confidence to identification of PAH sources. Almost two decades ago, recognition that PAH concentrations in sediments often do not correlate with ecological toxicity resulted in replacing “consensus standards” based on concentrations alone with equilibrium partitioning approaches, with the emphasis on exposure to bioavailable or bioaccessible PAHs. At the same time, measurement of bioavailability of specific PAH-containing materials has become a accepted adjustment to human health risk assessments. The recommendation of EPA’s Science Advisory Board to conduct toxicity testing on a variety of PAH-containing mixtures with an eye towards replacing the additive “Relative Potency Factor” approach reflects recognition that antagonism as well as bioavailability likely are important mediators of potential PAH risks. Recent work demonstrating the dominating importance of PAH source materials on partitioning indicates that properties in addition to individual PAH concentrations of different mixtures hold the key to assessing PAH bioavailability and risk.

745 PAHs in urban sediments of the Lower Rouge River “Old Channel”: fingerprinting, source characterization, robust statistical methods

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The global prevalence of polycyclic aromatic hydrocarbons (PAHs) in urban environments holds significant implications for contemporary public health and environmental remediation efforts. Various statistical methodologies are used in modern approaches to characterization of PAH contamination sources. The continued evaluation and improvement of these methods represents a central, enduring project in environmental forensics. From 2008 to 2014, hundreds of sediment samples were collected from the “Old Channel” segment of the Lower Rouge River (Detroit, MI) on behalf of the U.S. Environmental Protection Agency (EPA). Select samples were analyzed for 38 parent and alkylated PAHs via a modified EPA Method 8270. In this study, conventional ‘fingerprinting’ source characterization methodology, classical principal component analysis, and robust principal component analysis are used to describe the mixture, type, and magnitude of PAH contamination indicated by the analytical data. The advantages and limitations associated with each approach to PAH source characterization are explored through a discussion of the results generated by each method. Synthesis of PAH distribution analyses and interpretation of quantitative results contextualize the complex of PAH contamination sources observed within PAH forensics literature and contemporary data science and chemometric techniques.

746 Using PAH Compositional Analysis to Evaluate Dissolved-Phase Groundwater Discharge to Surface Water from Local Soil/Sediment Influence

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Groundwater discharge to surface water is a pathway of growing concern in evaluating the potential toxicity and/or bioavailability of polycyclic aromatic hydrocarbons (PAHs) from upland sources to aquatic resources. This potential pathway for surface water impairment is particularly relevant at former manufactured gas plant (MGP) sites, where coal tar dense nonaqueous-phase liquid (DNAPL) and high-concentration residuals can occur within close proximity (both upland and offshore) of shorelines where product and wastes were historically handled and transported. Because groundwater typically discharges to surface water close to the shoreline, nearshore sediment remediation may require mitigation of not only contaminant conditions in sediments but also in discharging groundwater. To design a remedy that addresses the groundwater pathway, it is essential to distinguish PAH loading via groundwater discharge from elevated PAH concentrations in pore water resulting from contact with contaminated sediments. PAHs from both pyrogenic (e.g., coal tar or creosote) and petrogenic sources contain compositional features that enable distinguishing dissolved-phase PAHs from either particles or NAPL entrained in a groundwater sample. Because pyrogenic NAPL compositions are very similar to dissolved-phase compositions, some prediction of phase-partitioning should also be used to support the interpretation. This case study from a former MGP site remedial investigation illustrates how PAH compositional analysis and predictive phase partitioning were used to identify PAH sources in groundwater and in pore water along a field-delineated groundwater discharge flowpath. Various compositional features were considered and compared with equilibrium partitioning predictions. The key features proved to be a combination of comparing concentrations and compositions in the groundwater/pore water with predictions, and comparing compositions (but not concentrations) with DNAPL and co-located sediments. Pore water PAH concentrations and compositions indicate that the PAHs are primarily influenced by the contamination of the sediments with which the pore water is in direct contact, rather than a dissolved-phase plume transported along a groundwater flowpath. The results were used to preclude the need for upland groundwater treatment or remediation, and to inform the reactive cap design for contaminated nearshore sediments.

747 Challenges in Analysis of Hydroxy PAHs in Urine

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Monohydroxy polycyclic aromatic hydrocarbons (OH-PAHs) are the major metabolites of PAHs. Despite their importance as biomarkers of PAH exposure, OH-PAHs are notoriously unstable. Consequently, countless studies have reported difficulties in the analysis of OH-PAHs. The present work sought to identify and remedy issues related to OH-PAH instability, in order to enhance analyte recoveries and obtain a robust method for elucidating PAH exposure in the general population. Stability experiments involving spiked water stored for 14 days at 20°C, in the presence of direct sun light, revealed >98% loss for all analytes. Solvent type, but not temperature played a significant role in analyte stability. The source of instability was identified as photo-induced oxidation. Analysis of urine samples obtained from mice fed with a PAH-contaminated diet revealed that >94% of each analyte was present in conjugated (glucuronated and sulfated) form. An investigation into the stability of the conjugated and free forms revealed the latter species to be the most stable. Overall, analyte instability was minimized by reducing exposure to light and through the use of an antioxidant and nitrogen atmosphere. The findings summarized in this work are relevant for those involved in biomonitoring of PAHs.

748 Review of Bioaccumulation and Bioconcentration Factors Used by USEPA in Derivation of 2015 Human Health Water Quality Criteria

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In July 2015 EPA released updated human health ambient water quality criteria (HHWQC). As part of that update EPA revised its approach to estimating bioaccumulation of substances into fish employing bioaccumulation factors (BAFs) in place of bioconcentration factors (BCFs) when supported by existing data and methodology. In January 2016 EPA released additional guidance providing more detail on the process followed to update bioaccumulation factors. This presentation has two goals. First, a review of that process to determine if it was applied correctly by EPA when developing HHWQC for PAHs and other highly metabolized compounds. Second, to the extent the process was not followed and alternative BAFs (or BCFs) should or could have been derived, the paper presents those alternative BAFs (or BCFs) and associated revised HHWQC for PAHs. This paper also provides a general overview of the process developed by EPA, evaluates its application on a national basis, and as necessary, recommends modifications that would improve the process.

749 Assessing PAH-related toxicity by spiking field-collected sediments with a site-specific contaminant mixture

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The sediments of Bear Creek near Baltimore, Maryland contain a complex mixture of organic and inorganic contaminants, including polycyclic aromatic hydrocarbons (PAHs). PAH concentrations measured in both whole sediment and sediment pore water have shown positive correlations with amphipod mortality during laboratory assessments of field-collected sediment. However, numerous other constituents, including heavy metals, have also shown positive correlations with toxicity at the site, making it difficult to determine whether PAHs are a driver of observed adverse biological effects within Bear Creek. To further examine the relationship between PAHs and observed toxicity within Bear Creek sediments, and to determine whether PAHs are a likely driver of toxicity at the site, a spiked sediment test was conducted using a subset of the suite of PAH compounds found at the site. Sediments were collected from two control sites that represent a similar sediment matrix to previously assessed contaminated samples from Bear Creek. Sediments were spiked with a mixture of 18 PAH compounds detected at the Bear Creek site, as determined by previous analysis of PAHs in total sediments and sediment pore water. Spike total PAH concentrations were selected based on observed concentrations of PAHs in site sediments that yielded toxicity results ranging from complete mortality to complete survival. Pore water from spiked sediments was analyzed by an antibody-based PAH biosensor that is sensitive to 3-5 ring PAHs, and sediments were used to conduct a ten-day acute toxicity test using the benthic amphipod Leptocheirus plumulosus. Results provide insight into the relationship between PAHs and sediment toxicity to benthic organisms at a specific site, while the study demonstrates the application of site-specific spiking experiments to sediments containing complex mixtures of multiple contaminant classes.

750 Individual PAH metabolites in English sole (Parophrys ventulus) bile from Puget Sound, WA, USA from the past 10+ years

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Polycyclic aromatic hydrocarbons (PAHs) are commonly found in the environment, particularly in urbanized areas or in regions with petroleum-related activities. PAHs pose risks to marine life, as these compounds are known to be carcinogenic and toxic, affecting survival, growth and reproduction of several species. Due to the ability of fish to quickly metabolize PAHs, it is crucial to assess the presence of metabolized PAHs in fish, in addition to the conventional parent (non-metabolized) PAH analysis, for a better understanding of the exposure to PAHs and their fate in fish. Biliary PAH metabolites can be found at relatively high levels in fish from areas where very low levels of parent PAHs are detected in abiotic samples or fish muscle, due to the fast metabolism and accumulation in bile prior to excretion. Moreover, during the excretion process, the PAH metabolites can be reabsorbed by fish and become more toxic than the parent PAH. Unfortunately, there are limited data on environmental levels of PAH metabolites in marine species, particularly from urbanized areas such as Puget Sound, WA. We recently developed a rapid method for accurately quantifying 27 individual hydroxylated PAH metabolites (OH-PAHs) in fish bile. The method consists of removing polar lipids and protein, hydrolyzing phase II conjugated OH-PAHs, solid-phase extraction, and analysis by LC-MS/MS. The method was validated using pre-spiked (efficiency) and post-spiked (matrix effect) tests and reference and control materials. Recoveries of spiked samples ranged from 60 – 129% (mean 97%, SD 15%). Using this method, we determined PAH metabolite concentrations in bile of English sole (Parophrys ventulus) from 3 urban sites of Puget Sound collected from 1997 – 2015. Several OH-PAHs were detected in 100% of the samples, except control site, with dihydroxydihydro forms of both low- and high-molecular weight PAHs being the most abundant metabolites. This distribution suggests of multiple sources of PAHs in to Puget Sound, with summed metabolite levels reaching over 5,000 ng/mL of bile. Our findings indicate that English sole, and potentially many other marine species, from Puget Sound are exposed to elevated levels of toxic and carcinogenic PAHs.

751 Toxicity in zebrafish of complex polycyclic aromatic hydrocarbon mixtures exposed to terrestrially-appropriate UV-irradiation

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Previously, we determined degradation kinetics for a mixture of environmentally-relevant polycyclic aromatic hydrocarbons (PAHs) derived from an EPA-designated Superfund site and denoted Supermix 10, when irradiated with accurate solar-emulating Ultraviolet light (UV). That initial study has been expanded to double the time scale and as well as examine the role of spectral perturbation in determinations of half-life and product formation. By manipulating filters on a xenon-arc source, three differing spectral compositions were established. They were characterized by UVA to UVB ratios of 20:1 (approximating solar flux), 10:1 (excess UVB) and 4:1 (excess UVB and UVC). Pairwise comparisons of the three spectral compositions show statistically significant differences in kinetics of degradation for all analytes within the mix between 20:1 and 4:1 ratio sources, for a constant UV flux. As predicted, only phenanthrene, an analyte whose absorbance spectrum drops precipitously near the border of UVA and UVB, showed a statistically significant increase in degradation rate between the 20:1 and 10:1 UV sources. Pairwise examination of four analytes irradiated to a defined dose individually as well as irradiated within the complex mixture showed consistent, but relatively small differences and demonstrated artifacts likely common to individual low-concentration irradiations. Source comparisons were extended to differential product formation by direct comparison of total ion chromatograms from 20:1 and 4:1 UV sources. To examine the toxicological implications of this UV-modified complex mixture, embryonic zebrafish were exposed to unirradiated Supermix 10 and Supermix 10 exposed to a series of UV doses corresponding to 2, 4 and 8 hrs of exposure. Unirradiated Supermix 10 toxicity is driven by benz(a)anthracene, pyrene and retene, three chemicals with relatively short half-lives of 29.4, 77.0 and 346 minutes respectively within our irradiation system. Our working hypothesis is that the relatively rapid degradation of these three drivers of toxicity will reduce toxicity of Supermix 10 relative to its unirradiated counterpart.

Soil and Water Contaminants: Evaluation, Biomonitoring and Abatement Bioindicators for Effective Management

752 Metal Concentrations in Urban Soils of Kumasi Metropolis, Ghana

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Ninety-six surface soil samples were collected using a 0.5 km x 0.5 km grid in areas where most of the commercial activities were concentrated in Kumasi, the second most populated urban area in Ghana, West Africa. The samples were analyzed for total metals using a field portable X-ray fluorescence analyser (FPXRF). A subset of the samples (38) was also analyzed by aqua-regia/ICP-MS. A comparison of the FPXRF data to the ICP-MS results indicated a strong linear correlation between the two methods for the majority of the metals (e.g., the Pearson Correlation coefficients ($p < 0.001$) for As, Cu, Pb, and Zn were 0.985, 0.894, 0.879 and 0.985, respectively. This indicated that the FPXRF is a useful tool for screening a large number of samples for potential human health risk assessment of metal exposure. Multivariate and geostatistical analyses of the data suggested that Cr, Cu, Fe, Ni, Pb and Zn in the soils were mainly derived from anthropogenic origin whereas As, Cd, Hg, Mn, V, and Sn were attributable to soil parent materials and atmospheric deposition. The concentrations of As, Cr, Cu, Pb and Zn in some of the samples exceeded environmental quality guidelines for residential land use such as the Canadian Council of Ministers of the Environment soil quality guidelines. The use of geo-accumulation, enrichment factor and pollution load indices indicated high contamination in some of the soil samples. To assess the potential health effects associated with ingestion of the metal contaminants in the soils an in vitro physiologically based extraction test was used to determine metal bioaccessibility. The mean metal bioaccessibility were As (14%), Cr (2.8%) Cu (47%), Pb (62%), Ni (24%) and Zn (73%). Based on the total metal concentrations, soil properties and bioaccessibility data the risk associated with exposure to metals (e.g., Pb, Cu and Zn) at some of the sampling locations were deemed high. Ongoing work includes detailed risk characterization and the development of a strategy for effectively communicating the identified risk and recommendations for limiting exposure to the identified metal contaminants.

753 Fates of some phenylurea herbicides in tropical soils: trends and correlations

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Fates of xenobiotics in tropical soils have received considerably little attention, despite the fact that approximately one half of the earth's population and roughly one third of its land mass are found in the tropics. In this work, adsorption and mobility of three phenylurea herbicides (Diuron, Linuron and Monuron) were studied in typical Nigerian soils. Eighteen top soils (majorly of Alfisol, Inceptisol and Entisol orders on the USDA taxonomy) were collected at different locations in the Southwestern Nigeria. Samples were comprehensively characterised for their physical-chemical properties, using standard methods, and soil mineralogy (powder and texture preparations) was determined with XRD. Eight of the samples were later selected for adsorption test, using batch equilibrium method. Effects of solution pH, temperature, concentration, and contact time were investigated; and also were sorption kinetics and isotherms. The samples were analysed for equilibrium concentration with HPLC-DAD at 250nm, and multivariate analysis of the data was carried out with R. Results indicated that the pH ranged from 5.37 – 7.54, the %SOM ranged from 0.92-6.97. %N ranged from 0.1 – 0.9, and CEC_{effec} ranged from 21.5 – 112 mmolc/Kg. Dithionate extractable oxides- Fe_d, Al_d and Mn_d ranged from 2.02-14.62, 0.11-0.64 and 0.16-3.13g/Kg respectively, while oxalate extractables- Fe_o, Al_o and Mn_o ranged from 0.20-1.86, 0.13 – 1.42 and 0.14- 2.32g/Kg respectively. Granulometry results showed that %sand ranged from 66-92, while silt+clay proportion ranged from 8-34%. XRD analysis of the powder samples revealed the presence of albite, microcline and quartz in all samples. Some samples contained sodalite and dolomite in addition. The semi-quantitative XRD analysis of the clay fraction revealed the dominance of kaolinite (41-93%), followed by illite (7-60%), with only three samples containing smectite (3-12%). Batch tests- though still ongoing, revealed strong positive correlations between sorption parameters and soil properties such as SOM, Mn_o, Fe_o, CEC, Mn_d, soil pH, Fe_d, and Al_d (in decreasing order of magnitude of r). K_d, K_{oc} and K_f ranged from 0.03-0.10, 2.29-5.34 and 0.07-0.19 respectively. Adsorption parameters decreased with increase in temperature while test solution pH showed no significant effect. The K_{oc} got in this study and the $t_{1/2}$ reported in literature will be used to assess the leachability of the pesticides in the soils, using the Gustafson Ubiquity Score (GUS).

754 Polychlorinated Biphenyls (PCBs) in Fish Fillet Tissue: Nationally/Regionally Representative Statistical Surveys of US Waters

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As of 2015, the USEPA has conducted probabilistic, nationally-representative assessments of polychlorinated biphenyls (PCBs) in fish tissue in U.S. lakes (2000-2003), twice in rivers 5^{th} order and greater (2008-2009 and 2013-2014) as part of National Rivers and Streams Assessments (NRSAs), and in nearshore waters in the Great Lakes as part of the National Coastal Condition Assessments (2010 completed, 2015 in progress). Not manufactured in the U.S. since 1979, PCBs are an extremely persistent group of organo-chlorine compounds that are toxic and carcinogenic, and readily bioaccumulate in fish, especially predatory species. PCBs are highly pervasive in fish, with the highest mean concentrations in the U.S. occurring in the Great Lakes; intermediate concentrations occurring in U.S. rivers, with those concentrations being significantly elevated in the Eastern Highlands eco-region relative to the rest of the country; and relatively lower concentrations occurring in non-Great Lake U.S. lakes. Availability of analytical results from the 2013-2014 NRSA allows us to compare PCB concentrations in fish tissue in these three sets of waterbodies (U.S. rivers 5th order and greater, Great Lakes, other U.S. Lakes), the extent to which observed concentrations of summed data for all 209 PCB congeners in fish tissue exceed relevant human health and ecologic thresholds in these waterbodies and in each of three eco-regions of the U.S., which also include the Plains & Lowlands and West & Mountains eco-regions.

755 Risk Assessment of Constant Use of Detergent in Fermentation Processing of Cassava in Southeastern Nigeria

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The study investigated, the risk associated with the use of detergents to speed up the processing of cassava which is a staple food in Southeastern Nigeria. Due to effects of climate related variables on the production and processing of cassava, rural dwellers have adopted the practice of using detergent to help the process of fermentation of cassava. First of all, field survey of the use of this crude processing method was carried out to establish the percentage distribution of rural dwellers practicing it. Cassava tubers were harvested in an unpolluted farm, carefully peeled, soaked in detergent water and allowed to ferment. The amount of detergent residue accumulated in the processed cassava was evaluated using the titrimetric method. Thereafter 24 albino Wistar rats were fed with the paste for 28 days to determine the toxicological effects of detergent residue on the organism. Catalase, superoxide dismutase, lipid peroxidation, ascorbic acid and glutathione were assayed. Liver and kidney parameters were also assayed. Results obtained showed that more than 70% of the rural dwellers (cassava processors) use detergent in fermenting cassava, 15% of them use other chemical substances to ferment it while only 5% of them do not add any chemical or additive to it. The dried cassava paste was found to contain between 0.019 to 0.177 mg/g anionic surfactant residue and between 0.058 to 0.177 mg/g cationic surfactant residue. Result of the enzyme assay showed that catalase, superoxide dismutase, alanine aminotranferase, aspartate aminotransferase and alkaline phosphatase were not significantly affected ($P < 0.001$); lipid peroxidation, ascorbic acid glutathione total protein, albumin, bilirubin, urea, uric acid, and creatinine showed no significant difference ($P < 0.001$). Care should be taken by rural dwellers, cassava processors should be educated on the risk associated with the use of chemicals to ferment cassava.

756 Use of nano iron (III) oxide (nFe3O4) for the remediation of organotin – TPT in Marine Wastewater System

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Triphenyl tin is present in antifouling paints where it is used to prevent the growth of fouling organisms on marine structures and vessels. TPT is a classified endocrine disruptor and can cause imposex in marine organisms. Thus it has the potential to affect the biodiversity of the marine environment. The potential effects stress the need to control its presence in the environment. Iron nano particle has generated some interest in recent years because of their unique characteristics which made them suitable for a number of applications including remediation of recalcitrant environmental pollutants. The removal of triphenyltin (TPT) from artificial seawater using nano iron (III) oxide (nFe_3O_4) was studied. The adsorption was investigated, including both equilibrium and kinetics. Equilibrium adsorption data were analyzed using Langmuir, Freundlich, Temkin and Dubinin–Radushkevich (D-R) isotherm models. Pseudo first- and second-order, Elovich, fractional power and intra-particle diffusion models were applied to test the kinetic data and $\Delta G°$, $\Delta S°$ and $\Delta H°$ were also calculated. The effects of adsorbent dose, contact time, pH, stirring speed and initial concentration of TPT were also studied to establish optimal conditions for the removal of TPT using the nano material. The optimal conditions established were applied to TPT removal from TPT – contaminated natural shipyard wastewater. The result showed that 95.37 % TPT was removed by the nFe_3O_4 which showed the use of nano iron as a possible route for the remediation of TPT contaminated wastewater before being discharge into the environment.

757 In vitro image-based phenotypic analysis with Liquid chromatography–mass spectrometry for evaluation of wastewater and river water quality

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Image-based phenotypic profiling was well applied for drug discovery through measurement of cellular phenotype that related on toxicity mechanism, which exhibit potential for toxicity identification evaluation. We developed an image-based phenotypic phenotypic analysis with human breast cancer MCF-7 cells to evaluate the impacts of whole wastewater and river water, and compared with conventional bioassay (e.g. cytotoxicity test) and non-target chemical analysis (UPLC-qTOF-MS) to evaluate the potential target chemicals. We used 13 cellular parameters (nuclear intensity, nuclear area, nuclear perimeter, nuclear major, nuclear minor, nuclear minor/major, nuclear form factor, cell intensity, cell area, cell major, cell minor, cell minor/major, cell form factor) to characterized cellular morphology, while defined "distance score" in PCA score plot to evaluate phenotypic variation. The "distance score" exhibited dose-dependently relation after 17β-estradiol (E2) exposure, which indicated the sensitivity of image-based phenotypic analysis. Whole water induced various effects of phenotypic variation such as cell body and nuclear shrink; synapse on the cell membrane; rhombus shape; enlarged cell body and nuclear that was well characterized with image-based phenotypic analysis. Compared with cytotoxicity of whole water, the "distance score" showed reduction from wastewater treatment influent to effluent benefited from the multiple toxicity-based parameters, which indicated the capability for toxicity reduction evaluation of image-based phenotypic analysis. To investigate the correlation between potential toxicant in water and cellular phenotype, cell morphology combining non-target chemical analysis (UPLC-qTOF-MS) were correlated using optimized potentials for liquid simulations (OPLS) analysis. We selected 10 phenotype-active chemicals which exhibited high potential as target chemicals in image-based phenotypic analysis. In image-based phenotypic analysis, multiple parameters exhibited information-rich, while phenotypic parameters provided toxicity-related and higher throughput endpoint for Toxicity identification evaluation. We believe that this method is a promising approach for environmental toxicant screening along with toxicity-phenotype-chemicals relations clarification and cell morphology database construction of environmental contaminants.

758 Risk assessment of continous discharge of mixtures of chemical subatances into an urban watershed in Owerri Southeastern Nigeria

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Abstract In this study, we used an efficient and economic science-based risk assessment approach to evaluate the impact of mixtures of unquantified concentrations of chemical substances being discharged into an urban watershed inOwerri, Imo State, Nigeria. Water samples were collected from the Nworie River, Owerri in South Eastern Nigeria. Physico-chemical characterization of water samples from upstream, midstream and downstream of the river was carried out using standard methods. Parameters studied included turbidity, colour, pH, phosphate, nitrate, BOD, COD, amongst others. Levels of selected heavy metals were also determined in the samples using atomic absorption spectrophotometry (AAS). In the static bioassay tests, five (5) batches of juvenile tilapia fishes, each having seven (7) fishes were exposed to the raw polluted water and filtered polluted water from the watershed as well as uncontaminated water (control) for twelve (12) to twenty-four (24) hours. The exposed juvenile fishes were observed for mortality after 12 h and 24 h exposure periods. Most of the physic-chemical parameters including heavy metals exceeded the WHO guidelines for surface water quality with zinc having the highest concentration in all the sampling locations, followed by lead and arsenic. In the bioassay, 60 – 73% of the juvenile fish died from the raw polluted water, and 15-20% for the filtered polluted water. No death was recorded in control. There is therefore, need for the mitigation and control of influx of chemical substances into this river ensure human and environmental health.

759 Assessment of the prevalence of Chromobacterium violaceum, in Domestic water sources in Imo State, Nigeria

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Chromobacterium violaceum, as opportunistic pathogen of extreme virulence, infects human through soil, contaminated – puncture wounds, food and water. It is a less common water-borne pathogen, but due to its high virulent nature, it becomes a great concern to public health and environmental scientists. Hence this research primarily aimed at monitoring and ascertaining the prevalence of this organism "Chromobacterium violaceum" in domestic water sources in a tropical environment and determining its antimicrobial susceptibility was embarked on. Twenty-four (24) water samples were aseptically collected from five water sources in Owerri (Otamiri River, Nworie River, Borehole, Tap and sachet water) into sterile bottles for this analysis. The water samples were transported to the Microbiology laboratory of the department of Medical Laboratory Science, Imo State University, Owerri within six (6) hours of collection and analyzed bacteriologically by pour plate method on Nutrient Agar for viable heterotrophic counts. Typical colonies of C. vidaceum were isolated and purified, on fresh sterile Nutrient agar medium. Thereafter, the antibiotic susceptibility test was performed using disc diffusion technique on sterile nutrient agar. Otamiri river source recorded the highest viable heterotrophic bacterial count ($7.4x10^5$cfu/ml), while Borehole water source recorded the least viable heterotrophic count ($1.5x10^5$cfu/ml). The prevalence of Chromobacterium violaceum in domestic water sources is 5(25%) and was isolated only from Otamiri River, sachet and borehole, among other sources. The antimicrobial profile showed that Chromobacterium violaceum is highly resistant to antibiotics. Hence, it is recommended that enlightenment campaign to educate the masses of the health risk associated with the use of water contaminated with this organism, while Government should vote more money into water ministry to fight this bug.

Mercury Fate and Biogeochemistry

MP001 Predicting methylmercury (bio)availability at the base of the aquatic food web

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Methylmercury (MeHg) toxicity is of particular interest in remote environments, far from point sources of contamination, where high levels of MeHg are accumulating in top predators. The ecosystem variables that control this sensitivity of food webs are not well known. MeHg concentration in water and uptake into the base of the food web is one key factor controlling mercury entry into food webs. To date, few studies have directly considered photodemethylation reactions in combination with physical attributes of aquatic ecosystems to predict where and when dissolved MeHg may be available. To address this research gap we have used numerous controlled and semi-controlled experiments that focused primarily on the quantification of the relationships between solar radiation exposures, dissolved organic matter (DOM), and MeHg within six freshwater lake systems. DOM photoreactivity measured through photobleaching of lake water within our study system was primarily driven by DOM concentration (quantified as dissolved organic carbon (DOC); R^2=0.94). A DOM photoreactivity manipulation experiment that used water from one lake did not show consistent prediction of photodemethylation rate constants. Photodemethylation rates tended to be higher in water collected during early summer, when in-situ DOM concentration was lower, than in water collected in late-summer or fall ($p < 0.05$). DOM concentration could explain 76% of variation in photodemethylation rates in an inter-lake comparison semi-controlled natural solar radiation experiments conducted over 1 week periods in summer and fall. By quantifying the photoreactivity of DOM and the interplay between DOM and MeHg photoreactions, we have developed a model for predicting photodemethylation potential and efficiency within the top 1 m^3 in oligotrophic dystrophic temperate lakes. These outcomes were then scaled up and used to calculate the overall photodemethylation potential in each of our six study lakes for comparison with MeHg concentrations in the corresponding food webs. This model may be appropriate for other aquatic ecosystems by simple standardization techniques depending on water quality characteristics such as DOM photoreactivity (structure), pH, and dissolved ionic species. Overall, this compiled body of work yielded a method for predicting mercury availability to food webs depending on environmental and physicochemical factors.

MP002 Does amino acid content explain methyl mercury concentrations in aquatic invertebrates?

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Methylmercury (MeHg) concentrations in aquatic invertebrates are known to be highly variable within and among systems. For example, in Kejimkujik National Park, Nova Scotia, Canada, Aeshnidae dragonflies showed a 3-fold difference in MeHg among lakes. Though the concentrations of MeHg in invertebrates are important predictors of MeHg in fish, the factors driving MeHg variability in invertebrates are less understood. In biota, MeHg is stored in proteins bound to the sulphur-containing amino acid cysteine. Our objective was to determine if the cysteine content of organisms varies within and between aquatic food webs and may be used to model MeHg variability among taxa and systems. Benthic macroinvertebrates from different functional feeding groups and zooplankton (bulk) were collected from six lakes in Kejimkujik National Park that are known to vary in physical and chemical characteristics and biotic MeHg. MeHg concentrations were measured and compared to protein bound cysteine (as cysteic acid) and 14 other amino acids (ultra performance liquid chromatography; UPLC). Multiple discriminant analysis revealed significant differences in the amino acid composition of taxa (Wilk's lambda, $p < 0.0001$) with cysteine as one of the primary discriminating variables. Cysteine content (per mg total protein) differed among invertebrate taxa, with the lowest concentrations in Limnephilidae caddisflies and the highest concentrations in zooplankton. Preliminary results also indicated that log MeHg (mg/kg dw) was significantly and positively related to cysteine within three of the four taxa ($p \leq 0.01$). These results suggest that cysteine content explains some of the among-taxa differences in MeHg concentrations and will improve our understanding of the factors that drive MeHg variability in aquatic food webs.

MP003 Interaction of Mercury with Thiols: Complexation and Enhanced Dissolution of Mercury Sulfide

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The biogeochemical cycling of mercury (Hg), a notoriously toxic contaminant of worldwide health concern, includes complicated transport and transformation processes that are regulated by a variety of environmental factors. The presence of thiol (-SH) containing substances in the environment, including low molecular weight (LMW) amino acids and natural organic matter (OM), has profound effects on the mercury speciation, mobility, and reactivity. The effect of thiol-containing substances on environmental behavior of mercury sulfide (HgS) has recently drawn much attention. Although often being regarded as one of the largest sinks for Hg in sediments, soils, and sulfuric waters due to its extremely low solubility, mercury sulfide can undergo enhanced dissolution in the presence of thiol-containing substances, which could act as an important process in controlling mercury transport, transformation, and bioaccumulation. In the presence of different thiol-containing substances, including cysteine, glutathione, and humic substances, the dissolution of mercury sulfide was investigated. It was found that thiol-containing substances could promote the dissolution of mercury sulfide, with varying enhancing effects depending on type of thiol-containing substances. The enhancing effect of thiol-containing substances on mercury sulfide dissolution was related to complexation of mercury with thiols. After dissolved mercury is released into the solution from mercury sulfide dissolution, the complexation of mercury with thiols could hinder the dissolved mercury being re-adsorbed back to the surface of mercury sulfide solids and thus enhance the dissolution.

MP004 The Influence of Avian Biovectors on Mercury Speciation in a Coastal Wetland

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Mercury is a persistent and bioaccumulative chemical that is present in many remote environments due to its ability to be transported long distances in the atmosphere, and to be deposited far from the original source (Sunderland and Chmura 2000). Wetland ecosystems are important "hot spots" for mercury in eastern Canada, providing anoxic environmental conditions that promote the bacterial methylation of mercury. Methylmercury is the most biologically available form of mercury and the form which biomagnifies in food webs (Gochfeld 2003). Seabird guano is a well-documented biovector for metals – including mercury – and nutrients, which may indirectly affect metal speciation (Choy et al. 2010). The site for this study, Big Meadow Bog (Brier Island, Nova Scotia, Canada) has a history of ditching in the 1950s, which changed hydrology significantly, resulting in colonization by 3000 pairs of herring gulls (Larus argentatus) in the 1980s. To quantify changes in mercury mobilization and speciation in response to this biovector, groundwater samples were collected from this site as well as a reference bog with similar geological and hydrological characteristics. The filtered samples were analyzed for total mercury, methylmercury, and water chemistry (pH, conductivity, anions, cations, and dissolved organic and inorganic carbon). Preliminary results show higher nutrients (nitrate, phosphate, and sulfate), total mercury, and methylmercury concentration when compared to the reference bog that is minimally impacted by avian biovectors. This elevated

availability of methylmercury could potentially pose a threat to the local ecosystem and wildlife population due to methylmercury's toxicity to living organisms (Akearok et al. 2010, Singh et al. 2011).

MP005 A mechanistic investigation of mercury reduction and volatilisation in soil

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Mercury (Hg) is a ubiquitous, toxic and bioaccumulative environmental contaminant that undergoes reaction to change speciation and environmental fate. Some areas, like the Oakridge National Laboratory Y-12 National Security Complex (TN, USA), have experienced historical contamination of surrounding soils with Hg, and remediation of such sites has been complicated by poor mechanistic understanding of Hg reactions. Specifically, non-volatile Hg species in soil can undergo reduction to form volatile Hg(0), which can then be lost to the atmosphere; however, the mechanism of this reaction is not known. This work used a computational study, coupled with laboratory experiments to propose one mechanism for reduction of one environmentally relevant Hg species, mercury (II) chloride ($HgCl_2$), in soil-like environments. Computational modelling using Gaussian software suggests that $HgCl_2$ reduction might include a unimolecular dissociation driven by scissoring of the chlorine bonds to form Hg(0). Based on the energy of excitation suggested in these computational results, we hypothesized that UVB radiation would provide the energy necessary to reduce $HgCl_2$ to Hg(0) via photolysis, without requiring a secondary electron donor. To test this, clean silica sand was spiked with an aqueous solution of $HgCl_2$, and exposed to full spectrum radiation, while the flux of Hg(0) from this sand was quantified over time. Laboratory flux results show some unexpected variability that may indicate factors in addition to radiation (eg/atmospheric composition) could be affecting $HgCl_2$ reduction in these sand samples; as a result, experiments are ongoing in an attempt to discern the nature of these effects.

MP006 The influence of chloride concentration on Hg photochemical reduction in melted Arctic snow

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Mercury contamination in Arctic snow is a cause for concern due to springtime deposition and melt that may transport mercury to local aquatic environments. Chloride (Cl^-) content has been linked to snowpack Hg concentrations in the Canadian high Arctic, where higher Cl^- snow has higher Hg concentrations, which may mean greater Hg movement to aquatic environments with spring snow melt. Hg in frozen or melted snow may also undergo photoreaction, which will alter the Hg load in the snow/snow melt; however, the effects of Cl^- concentration on photoreactions of Hg in Arctic snow/snow melt have not been determined. A laboratory study was undertaken to quantify the effects of Cl^- concentration on Hg photoreduction kinetics in Arctic snow, with the goal of providing predictive equations that could be implemented into Hg fate and transport models. Snow was collected from Alert, NU, Canada from 9 to 11 May 2014 into 2.2L Teflon bottles, and melted before being re-frozen and shipped south for analysis. Samples were thawed and spiked with 0 – 10 ppm added Cl^- (as NaCl). Samples at each Cl^- concentration were irradiated in triplicate at 4°C, with UV (280 – 400 nm) radiation from 1.26 – 5.78 $W \cdot m^{-2}$ for 24 – 48h in a LuzChem photoreactor. Photoproduced Hg(0) was quantified using a Tekran 2537, and pseudo-first order kinetic parameters, photoreduction rate constants (k) and total photoreduced Hg amounts ($Hg(II)_{red}$), were determined for each UV/Cl^- treatment. Relationships between Hg photoreduction kinetics, Cl^- concentration and UV intensity were derived. With such relationships, existing Hg fate and transport models can better predict Hg reduction dynamics in the Arctic where snowpack Cl^- concentrations can be very spatially variable, and Cl^- loadings to snow are predicted to increase as changing climate conditions continue to influence Arctic sea ice cover duration and extent.

MP007 Evaluation of Fish Length to Methyl Mercury and PCB Relationships--Teasing Out Interactions to Support Risk Management

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Methyl mercury (MeHg) and polychlorinated biphenyls (PCBs) are being monitored in fish tissue at the Berry's Creek Study Area (BCSA), a Superfund site located in a side embayment of the Hackensack River in the Meadowlands, New Jersey. Mummichog (Fundulus heteroclitus) and white perch (Morone americana) have been monitored since 2009 for tissue concentrations of MeHg, PCBs, and other chemicals of potential concern. Levels of MeHg in fish are affected by a number of variables such as environmental methylation rates, bioaccumulation in prey, fish movement and growth rates, and fish population age structure, all of which can change annually. In systems where exposure is variable because of environmental chemical concentration gradients and fish movement, control for species size and understanding of other biotic factors is important for establishing a baseline data set for long-term monitoring. At large, contaminated sites, spatial refinement of bioaccumulation and risk patterns is critical to the success and cost effectiveness of risk management strategies. The objectives of this analysis are to quantitatively characterize the relationships between white perch and mummichog MeHg and PCB concentrations as a function of fish length/weight and to evaluate interactions among environmental compartments in order to establish a baseline data set for risk characterization. A correlation analysis was conducted for white perch and mummichog length/weight and MeHg and PCB concentrations; significant bivariate relationships were further evaluated to develop regressions using general linear models that included co-variables of sampling year, geographic reach, lipid content, tissue type, and sex. Correlations were found between mummichog length and MeHg tissue concentrations, but linear fits and r2 values were poor. Correlations were also found between white perch length and MeHg and PCB tissue concentrations. However, white perch lengths varied significantly among sampling years and thereby confounded interpretation of trends in MeHg and PCB tissue concentrations, preventing the development of meaningful regression relationships for the site. Therefore, to support long-term monitoring needs, sampling strategies were altered to focus on the most prevalent size class of white perch in the BCSA, not on only the largest white perch.

MP008 Numerical Water Quality Criteria Derivation to Protect the Florida Panther Using the Everglades Mercury Example

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The Florida panther (Puma concolor coryi) is listed as an endangered species under the Federal Endangered Species Act (ESA) and is Florida equivalent statute. This requires that the responsible agencies restore and protect the quantity an quality of the habitat and connecting corridors using a srategy set forth in the ESA-mandated Florida Panther Recovery Plan (FPRP). Unfortunately, some portions of South Florida considered by the U.S. Fish and Wildlife Service and its Florida counterpart to be suitable Florida panther habitats have a methylmercury (MeHg) contamination problem. This represents a toxic chemical barrier to full utilization of otherwise suitable habitat by Florida panthers that forage on prey linked to the aquatic food chain. The FPRP fails to take this toxicological barrier into account, beyond mention of a need to continue monitoring Florida panther blood and fur for toxic substances. This is a fatal flaw in the FPRP. It has been compounded by the failure of the U.S. Environmental Protection Agency to promulgate a MeHg Water Quality Criterion (WQC) and related Total Maximum Daily Load (TMDL) under the Clean Water Act to ensure Florida panther protection in the absence of timely promulgation by the State of Florida. Instead, EPA Region 4 approved a Florida Statewide Mercury TMDL Plan that sacrifices the human use of the Everglades as a sport fishery, let alone fish-eating wildlife that consume large fish at higher rates in proportion to their body weight than humans. This is also true of their opportunistic predators. The Florida panther is linked to the aquatic food chain via its consumption of raccoons, otter and juvenile alligators when its preferred prey species are scarce or inaccessible as a consequence of unsuccessful competition with the fitter or a physical barrier such as high water levels. A fish-equivalent WQC protective of the Florida panther can be derived using a series of linked steady-state bioaccumulation models, reasonable assumptions about predation preferences for raccoon, otter and juvenile alligator, their predation preferences for various fish, amphibian, and shellfish species, and reasonable values for representative bioaccumulation factors in the Everglades aquatic food chain. When that is done, Florida's TMDL Water Quality Target of 0.3 ppm in fish flesh cannot be demonstrated to be fully protective of the cognitive function of Florida panthers forced to forage on prey linked to the Everglades aquatic food chain.

MP009 Effects of Coastal Managed Retreat on Mercury Biogeochemistry

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We investigated the impact of managed retreat on mercury (Hg) biogeochemistry in the Bay of Fundy, which is subject to diffuse contamination with Hg. We visited Beaubassin Research Station on the Chignecto Isthmus between Nova Scotia and New Brunswick and collected sediment cores from an area of land that was due to be inundated with seawater after a failing dyke was intentionally breached. We returned two years later, re-sampled the dyke cell and also collected sediment cores from an adjacent mudflat and a salt marsh. We analysed the total mercury (THg) and methylmercury (MeHg) concentrations of the sediments in the cores. We discovered that the concentration of THg in the sediment doubled after the dyke was breached due to the deposition of fresh sediment that had a smaller particle size, and higher pH. The concentration of MeHg was 27% lower in the sediments after the dyke was breached. Because we found greater concentrations of THg and lower concentrations of MeHg after the sediments were inundated we can conclude that the reduction in MeHg was due to a low bioavailability of Hg to the sulphate reducing bacteria capable of methylating Hg. This may have occurred due to higher organic matter levels, greater sediment pH, and higher ionic strength. Overall we did not find any evidence to suggest that coastal managed retreat resulted in an elevated risk of Hg methylation during the first year after inundation. As the sediment becomes vegetated, increased activity of Hg-methylating bacteria may accelerate Hg-methylation rate.

MP010 The effect of solid phase sorbent materials on the leachability of mercury from contaminated soils

C. Miller, Troy Univ / Biology and Environmental Sciences; S. Lukjan, Troy Univ / Chemistry; J. McCurdy, Troy Univ / Biology and Environmental Sciences; A. Johs, Oak Ridge National Laboratory; B. Robertson, Alabama State Univ / Biological Sciences

Streambank soils within the East Fork Poplar Creek (EFPC) watershed have elevated concentrations of mercury (Hg) as a result of historic use and discharge of Hg into the system associated with activities at the Y-12 National Security Complex. Mercury can leach from these soils as a result of water level fluctuations and erosion of stream banks resulting in the soils acting as a source of Hg to the creek. Laboratory experiments were conducted to examine the amount of Hg leaching from two soil samples collected from EFPC and a soil sample collected from Hinds Creek, an uncontaminated creek used as a reference site. For these batch laboratory studies the amount of mercury leaching from the soils when exposed to artificial creek water, consisting of a calcium nitrate solution, or actual creek water was examined for two weeks with samples collected every 3-4 days. After two weeks, solid phase sorbents were added to some of the samples and the amount of Hg leaching from the soils with and without sorbent addition was examined for an additional two weeks. The concentration of Hg in the reference soil was 0.028 µg/g and the two soils from EFPC had Hg concentrations 100 and 10,000 times greater than the reference soil. After the two week leaching period with artificial creek water, the concentration of Hg in the aqueous solution was 7 ng/L for the reference site. The aqueous phase concentration from the treatments containing EFPC soils were 100 and 1,000 times greater than the reference soil treatments. The solid phase sorbents that were tested include Thiol-SAMMS®, Organoclay™ PM-199, Organoclay™ MRM, SediMite™ and a biochar. The results from this laboratory study will help evaluate the usefulness of solid phase sorbent materials as a remedial option for reducing the amount of Hg leaching from soils into EFPC.

MP011 Developing a Centralized Mercury Knowledge Sharing Platform Using Emissions, Fate and Exposure Data

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The global agreement reached in 2013 regarding Mercury is a milestone in the chemicals management of pollutants. The so-called Minamata Convention has been signed, and is now being ratified by many countries (27 at the time of writing). Critical to achieving the goal of protecting human health and the environment from mercury, is the grounding of action in evidence, which in turn relies on sound scientific information about emissions, fate and impacts, typically captured in scientific journals and other literature often not accessible to other stakeholders. To develop regional and local knowledge bases for management and evidence based decision-making requires, in part, the ability to capture, synthesize, analyze and visualize data at varying scales and timeframes. It is also essential to facilitate understanding among global governance mechanisms, financiers, regulators and other stakeholders on how mercury emission reductions and site–specific projects, associated with implementing provisions of the Convention, affect the broader exposure and risk profiles of human populations and ecosystems. This initiative to develop a centralized Mercury Platform, which is in its first phase, attempts to create and manage an interoperable platform, deployed via UNEP Live, for data upload and download as well as knowledge sharing through information synthesis and facilitation of Communities of Practice comprising expert and critical stakeholder groups. A potential user survey was conducted in early 2016, targeting scientists, Minamata Convention focal points, stakeholders and SETAC members and drew responses from >400 individuals from different geographic regions throughout the globe. The term "knowledge sharing" is a deliberate attempt to move beyond traditional web-based delivery of quantitative but un-interpreted information. This will be achieved through user--mediated requests for data synthesis and visualization as well as implementation of standardized data protocols and delivery of meta-data as a component of shared output. This is being done through a demonstration pilot hosted at the UNEP Live platform, using the Biodiversity Research Institute's Global Biotic Mercury Syntheses database on selected biota. A key element of knowledge creation is the incorporation of pre-set or user-defined ways to provide insights and answers, not just data. The foreseen critical elements of a more full scale deployment of the platform will also be presented.

MP012 A Mercury Impacted Aquatic Food Web Based on Empirical Data

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Historical mercury (Hg) releases occurred at a textile manufacturing facility on the South River, Virginia, resulting in increased Hg concentrations in biotic and abiotic media, which have not declined over the past thirty years, as originally expected. Effective remediation of the Hg contamination requires a clear understanding of both migration, and complete exposure pathways. This model is further complicated by the unique nature of the biogeochemical fate of mercury in the environment, along with the relationships of discrete linkages among abiotic and biotic endpoints. In addition, the remedy must include a robust monitoring program, informed by these pathways. As such, good quality empirical data are essential to ensure primary and secondary sources are being remediated. This presentation will summarize the characterization and assessment of the aquatic food web associated with a portion of the South River watershed based on comprehensive empirical data collected over more than a decade. These data sets formed the basis for the development of the monitoring scheme that will provide input to an adaptive management framework to assess the effectiveness of the proposed remedy.

MP013 Survey of mercury in the fur of Bats (Chiroptera) of Coastal South Carolina

N. Roach, Florida Gulf Coast Univ; D.G. Rumbold, Florida Gulf Coast Univ / Depart of Marine and Ecological Sciences

Exposure to methylmercury (MeHg) has been widely overlooked in insectivorous organisms due to the perception, which has been shown repeatedly to be incorrect, of reduced exposure by low trophic level organisms. Bats may be at risk of MeHg exposure due to their high metabolic rates, relatively low caloric content of their prey, link to aquatic food webs, and their long lifespan, of up to twenty years. Bats eat a wide variety of insects, some of which have aquatic larval stages which gain MeHg from their environment before becoming a flying adult insect. Accordingly, fur was collected from bats during a "Bat Blitz" in Beaufort County, South Carolina over three days in July 2015. This area of South Carolina is colloquially called the "low country" and characterized by the small elevation gradient, large tracts of marshlands, and meandering streams. Total-Hg in fur ranged from 1.5 mg/kg to 34.8 mg/kg in the Seminole Bat (Lasiurus seminolus, n=117) and from 3.5 mg/kg to 78.0 mg/kg in the Big Brown Bat (Eptesicus fuscus,n=40). Patterns in fur-Hg related to sex, maturity, forearm length and location will be presented and discussed in the context of results of surveys of bats from other areas reported in the published literature. To our knowledge, this is the first survey of Hg in bats in the United States south of Virginia.

MP014 Mapping gaseous mercury concentrations around an abandoned mine in Italy using passive air samplers

D. McLagan, Univ of Toronto Scarborough / Chemistry; F. Monaci, Univ of Siena; H. Huang, Y.D. Lei, C.P. Mitchell, Univ of Toronto Scarborough / Dept of Physical and Environmental Sciences; F. Wania, Univ of Toronto at Scarborough / Physical and Environmental Sciences

The Abbadia San Salvatore mercury (Hg) mine (Central Italy) was the largest mine of the Mount Amiata Hg ore district and the third largest site of Hg production during the 20th century. Despite the cessation of operations in 1983 and recent remediation work, the mine remains a major source of Hg to the environment, especially the atmosphere. To map the atmospheric dispersion of gaseous Hg in the region we employed our recently calibrated passive air sampler for gaseous Hg. Sampling has been completed across two 7-by-7 sampling grids: a fine-spatial scale (~1km^2 total area) around the mine itself (week long deployments in October 2015 and June 2016) and a coarser grid (~50km^2 total area) across the western slope of Mt. Amiata (four 3-month deployments in 2015/16). The vertical concentration gradient of gaseous Hg was assessed by placing samplers at 2-4 m intervals above the ground on several taller structures within the mine site. Finally, uptake experiments were conducted in the Pacific Furnace and Carpentry buildings, both known to have very high gaseous Hg concentrations, to ascertain the maximum amount of Hg that the samplers can take up and provide increased temporal resolution data at the two sites. The new passive samplers provide the first time-averaged, highly spatially-resolved, and spatially concurrent mapping of gaseous Hg in and around a Hg mine. During the October fine-scale deployment, a strong concentric pattern was observed, with concentrations declining rapidly from the centrally located Pacific Furnace building. Concentrations of gaseous Hg at the Pacific Furnace (up to 7000 ng m^{-3}) and other locations across the mine were in good agreement with other studies that have conducted instantaneous, but non-concurrent gaseous Hg measurements at the site. The vertical gaseous Hg profiles just outside the Pacific Furnace site showed a very strong, decreasing logarithmic relationship with height above the ground, suggesting substantial evasion from an elevated surface source. Samplers did not reach equilibrium at the high-concentration sites despite accumulating up to 3100 ng of Hg over a week of sampling. The temporal variation in this data also correlated well with temperature and a high rainfall event that occurred during the deployment. Analysis of coarse-grid samples is ongoing and results should provide a clear picture of the mine's impact on atmospheric Hg in the region.

MP015 Analysing Nova Scotia lichens for mercury content

C.H. Saunders, A. Walker, Acadia Univ / Biology Dept; R. Cameron, Nova Scotia Provincial Government / Dept of Enviroment; N.J. O'Driscoll, Acadia Univ / Dept of Earth and Enviromental Science

Many lichens (a symbiosis of green algae or cyanobacteria and fungi), being epiphytic upon plant substrates, are known to be excellent air pollution indicators. A variety of lichen species are known to sequester airborne chemicals, including heavy metals such as mercury and are thus ideal biological indicators of such pollutant levels in the natural environment. Currently the spatial patterns of mercury associated with the lichens of Nova Scotia is largely unknown. This study will quantify spatial relationships in mercury adsorbed to lichens across Nova Scotia. Lichens in the genera Usnea and Hypogynmia were collected from sitesacross the province, including from long-term lichen monitoring plots. Samples were dried at air temperature, cryoground and analysed for total mercury by thermal degradation, gold amalgamation- atomic absorbance using a Nippon Mercury Analysis System (MA-2000). Geographic Information Systems will be used to produce mercury concentration gradient contour maps for the province in order to identify areas of high mercury contamination. Additionally, lichens of Nova Scotia are poorly represented in GenBank, and DNA barcoding of select specimens will be conducted, and the findings made available to the scientific community.

MP016 Mercury Concentrations in Apple Snails and Endangered Snail Kites Located in Florida, USA

C. Ortega-Rodriguez, Texas Christian Univ / Dept of Biological SciencesInst of Applied Science; B. Soulen, Univ of North Texas; R. Fletcher, E. Robertson, Univ of Florida IFAS; A.D. Sowers, US Fish and Wildlife Service; A.P. Roberts, Univ of North Texas / Dept of Biology Inst of Applied Science

Metals contamination in a freshwater gastropod, the apple snail (Pomacea sp.), poses a risk to surrounding terrestrial consumers who rely upon them as a food source, such as the endangered snail kite (Rostrahamus sociabilis). Mercury (Hg) is a metal contaminant found in nearly all aquatic systems. However, concentrations of Hg in apple snails and snail kites have not been extensively studied. Here, we determined concentrations of Hg (total Hg, Hg2+, and MeHg) in the native Florida apple snail (P. paludosa), the invasive island apple snail (P. maculata) and juvenile snail kite feathers to examine the relationship between Hg contamination in apple snails and juvenile snail kites. Apple snails and kite feathers were collected from central and southern Florida. Whole body total Hg concentrations for native apple snails was 47.67 to 205.11 ng/g dw, and ranged from 16.71 to 355.99 ng/g dw in invasive apple snails. Most of the total Hg in native and invasive apple snails was MeHg, which accounted for 70-99% of total Hg. Mean feather total Hg concentrations in snail kites ranged from 178.8 to 411.6 ng/g across sites. There was a positive correlation (r^2 = 0.83) between mean total Hg concentrations in apple snails and snail kite feathers across sampling sites. This study is the first to examine the relationship between Hg contamination in apple snails and snail kites and suggests that apple snails may provide a significant route of dietary exposure to MeHg in kites.

MP017 Dried Blood Spots as a novel tool for methylmercury exposure assessment

A. Santa, McGill Univ / Natural Resource Sciences; N. Basu, McGill Univ / Dept of Environmental Health Sciences

Methylmercury is a global contaminant of concern that is being addressed worldwide via the UNEP Minamata Convention. Dietary exposure to methylmercury primarily affects the central nervous system, while prenatal exposure may result in developmental deficits. Mercury levels in blood is a key biomarker for methylmercury exposure in humans and wildlife. However, the use of whole blood as a biomarker possess technical and logistical challenges such as the need for trained personal, adequate sample volume, and cold-chain transport and storage. Some of these challenges may be overcome with dried blood spots (DBS), which have been incorporated into major international data collection efforts (e.g., newborn screening) for over 50 years. The aim of this study was to develop a method to measure MeHg in DBS. DBS were created using human blood MeHg standard reference material (SRM) from the Institut National de Santé Publique du Québec. Three SRMs were used to encompass a range of MeHg concentrations: low 3.28+/- 0.11 parts per billion (ppb), medium 8.68+/- 0.26 ppb, and high 37.21+/- 0.02 ppb. Two, 3 mm sub-samples (6.5 microliters of blood) were digested using alkaline digestion and analyzed with a Tekran 2700. Preliminary results indicate that concentrations of MeHg fall between the expected ranges. Application of this method to wildlife surveillance programs and human biomonitoring endeavours are yielding fruitful results, which will be summarized in the presentation. Taken together, these studies are showing that DBS can be used as a tool to assess MeHg exposure with high accuracy and precision.

MP018 Nutrients and Hg cycling in Long Island Sound embayments

Z. Baumann, Univ of Connecticut / Marine Sciences; N. Mazrui, X. Shi, W. Huffman, R.P. Mason, Univ of Connecticut

The concentration of nutrients in estuarine waters has been hypothesized to influence the magnitude of methylmercury (MeHg) bioaccumulation. A conceptual model developed by Driscoll et al. 2012 suggests changes in nutrient loadings can alter MeHg bioaccumulation in resident biota through several pathways. Data to support the model is, however, yet to be published. This study covers sampling, during the spring and summer of 2016, of water, sediment and biota including shellfish, zooplankton and small forage fish from 5 embayments, known to receive different nutrient concentrations within Long Island Sound. The concentration of total mercury (HgT) and MeHg were measured in water, sediment and biota samples. Concentration of nitrate, nitrite, ammonia and phosphate in the water and pore water collected from these locations were also measured. In addition, we measured dissolved sulfide in pore water as sulfide substanially impacts Hg geochemistry. This presentation will focus on the gradient in nutrient concentrations in seawater and sulfide in pore water and how they influence mercury (Hg) biogeochemistry, especially the production of MeHg in sediments and MeHg bioaccumulation into resident biota. Thus far, our findings show differences in the fraction of HgT occurring as MeHg (% MeHg) in porewater collected from surface sediments, which ranges from 1 to 14%.

MP019 Mercury in Fishes from Yosemite (CA), Mount Rainier (WA), and Rocky Mountain (CO) National Parks: Potential Drivers and Ecological Risk

C. Flanagan Pritz, National Park Service / Air Resources Division; C. Eagles-Smith, USGS / FRESC; J.J. Willacker, USGS / FRESC; M. Lutz, USGS-WRD / Water Resources Division; M. Tate, USGS

The National Park Service (NPS) safeguards over 400 special places for the protection of unique natural resources and scenic beauty. Although celebrated as some of the most pristine ecosystems, recent studies have documented the presence of mercury in fish from NPS units across the western United States, including Mount Rainier (MORA; WA), Rocky Mountain (ROMO; CO), and Yosemite (YOSE; CA) national parks. Even protected areas, such as national parks, are subjected to mercury contamination because it is delivered through atmospheric deposition, often after long-range transport. Approximately 1,200 fish (primarily Salmonidae) were sampled from 52 subalpine lakes and rivers between 2003–2012 in MORA, ROMO, and YOSE, and analyzed for total mercury concentrations. Preliminary results indicate that mercury concentrations in fish varied substantially among and within parks, suggesting that landscape factors may be particularly important determinants of mercury risk in the parks. Basin delineations and landscape models were utilized to determine significant drivers in fish mercury concentrations, such as atmospheric deposition and dissolved organic carbon (DOC). Although mercury concentrations in most fish were low, at some sites they exceeded health thresholds for potential impacts to fish, birds, wildlife, and humans. Ongoing studies aim to further characterize mercury in fish from eastern national parks, evaluate the utility of dragonfly larvae as a biosentinel for mercury in aquatic food webs, and assess mercury source attribution via mercury stable isotope markers. With a strong foundation in research, the NPS strives to understand how best to minimize air pollutants in park ecosystems, including airborne contaminants like mercury where concentrations in fish challenge the very mission of the national parks to leave resources and wildlife unimpaired for future generations.

MP020 Mercury and metals deposition in Flin Flon Manitoba and the Experimental Lakes Area Ontario (Canada): a multi-lake sediment core reconstruction

J. Wiklund, Environment and Climate Change Canada; J.L. Kirk, D.C. Muir, Environment and Climate Change Canada / Aquatic Contaminants Research Division; D.C. Muir, M. Evans, F. Yang, J. Keating, M. Parsons, Environment and Climate Change Canada

Metal ore smelting and refining has historically been a major source of Hg globally, accounting for ~76% of atmospheric anthropogenic Hg emissions between 1850-2008. However, there is a great deal of uncertainty surrounding current and historical Hg deposition and re-emission rates associated with this source. We will present a landscape-scale multi-lake approach to reconstruct the history of Hg deposition sourced from human activities in two remote regions of Canada: the Experimental Lakes Area (ELA), Ontario and Flin Flon, Manitoba, which has been a copper and zinc mining and processing centre since the 1930s and Canada's largest Hg emitter until 2010, when the smelter was closed. 14 high fidelity lake sediment cores were collected from the ELA and Flin Flon regions in 2009-2010. Cores were dated using ^{210}Pb methods, then analyzed for concentrations of Hg. Results show that records of anthropogenic Hg flux and inventory were remarkably consistent among the 5 ELA lakes, but varied by 2 orders of magnitude among the 9 Flin Flon lakes. For the Flin Flon region, we integrated the strong exponential relation between anthropogenic Hg inventory (μg/m^2) and distance from point source to estimate changes in Hg deposition over 5-year time steps within 50 km of the smelter. We estimate that 64±16 tonnes of locally sourced anthropogenic Hg were deposited on the landscape over the ~80 years of smelter operations, which is ~20 times greater than the quantity that has accumulated within 50 km of the ELA since ~1860. Deposition to the Flin Flon landscape accounted for ~11% of estimated emissions until the 2000s, when smelter releases were reduced >10-fold and observed deposition exceeded smelter releases. These results suggest that landscape re-emission of legacy Hg will remain a local and global Hg source for unknown time-scales. Analyses of 45 elements in these same sediment cores demonstrates enrichment of numerous metals/metalloidsover the pre-~1860 baseline in both the ELA and Flin Flon regions. For example, we estimate that 99749.24 Tonnes of metals composed of Ag, As, Bi, Cd, Cu, Ga, Pb, Sb, Se, Tl, Zn, and Te was deposited to the landscape within 50 km of the Flin Flon smelter over its operative career. This represents ~55% of estimated dust releases to air for roughly the same period, highlighting the differences in transport/deposition pathways among Hg and other metals.

Status and Trends of the Landscape-Scale Mercury Problem in South Florida and the Everglades

MP021 Mercury Concentrations in Feathers of Adult and Nestling Osprey (Pandion haliaetus) from Coastal and Freshwater Environments of Florida

D.G. Rumbold, Florida Gulf Coast Univ / Depart of Marine and Ecological Sciences; K. Miller, T. Dellinger, Florida Fish and Wildlife Conservation Commission; N. Haas, Florida Gulf Coast Univ

We determined mercury (Hg) concentrations in feathers of osprey (Pandion haliaetus), both nestlings (n=95) and adults (n=110), across peninsular Florida and the Florida Keys during February-August 2014. Feathers plucked from nestlings, aged 3 to 7 weeks, contained Hg concentrations that ranged from 0.338 mg/kg to 45.79 mg/kg and averaged 6.92 ± 7.58 mg/kg (mean ±1SD). Feathers shed from adults contained significantly higher concentrations ranging from 0.375 mg/kg to 93.65 mg/kg, with an average of 17.8 ± 16.1 mg/kg. These levels were in the upper range of previously reported feather Hg concentrations of osprey and clearly show that Florida continues to have Hg hotspots that are elevated compared to many other regions. While these concentrations did not exceed levels previously reported in osprey from heavily Hg contaminated areas that showed no evidence of reproductive impairments, we cannot rule out potential individual-level effects to highly exposed nestlings after fledging.

MP022 Recreating the Everglades: laboratory modeling of in ovo mercury exposure in the American alligator

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The Florida Everglades are one of the most unique and biologically diverse ecosystems on the planet, and home to a variety of endangered animals. The Everglades are also known to accumulate mercury at much faster rates than similar environments in other locations, due to a 'perfect storm' of environmental parameters. These factors make the Everglades an ideal place to study many species-specific responses to mercury exposure; however routine sampling in the Everglades is difficult due to the system's remote location, challenging landscape and required resources. In this study, we utilized the American alligator, a reptilian sentinel with an extensive range throughout the southeastern United States to model mercury exposure in ovo in an Everglades apex predator. Using a routinely monitored population of alligators in coastal South Carolina, we calculated total mercury concentrations in adult blood, egg yolk, and embryos and generated a formula to model this relationship in alligator populations at other locations using an adult female blood sample. We then conducted a laboratory dosing study that reflected the expected egg yolk and embryonic concentrations of total mercury for American alligators in the Everglades. This study utilized two clutches of live eggs collected from a site northern Florida where low concentrations of mercury have been previously measured in adult alligators. Total mercury was measured in a sample of eggs (n = 3) from both clutches prior to dosing, taking into account intra-nest variation observed in previous studies. Eggs from both clutches were combined and then randomly divided into three groups (control/natural dose, low dose, high dose) and dosed by topical application with methylmercury-cysteine at stage 12 of embryonic development. Eggs from each group were analyzed for total mercury at 24hr, 48hr, 7d and 14d after dosing to determine the rate of mercury transmission through the eggshell. The remaining eggs were incubated until stage 27, and then embryonic blood samples were collected and analyzed for total mercury (THg). The samples from 24hr, 48hr, 7d and 14d after dosing suggest that much less than the published 33% of topically applied chemicals transfer through the eggshell. This study demonstrates the utility of modeling for lab-based environmental contaminant research, high intra-clutch variation in THg, and the limitations of topical application as a chemical administration technique in egg dosing studies.

MP023 Assessment of total mercury in sentinel estuarine fishes of the Florida Everglades and adjacent coastal ecosystems

D. Tremain, Florida Fish and Wildlife Conservation Commission / Fish and Wildlife Research Inst; D. Adams, Florida Fish & Wildlife Commission / Fish and Wildlife Research Inst; D.W. Evans, NOAA Center for Coastal Habitat and Fisheries Research

Mercury is a persistent toxicant of concern in south Florida that can bioaccumulate in aquatic food webs to levels that are harmful to human and ecosystem health. Proposed efforts to restore hydrological flow in the Florida Everglades may alter the spatial distribution and temporal trends in mercury bioaccumulation in south Florida fish populations. Monitoring of mercury concentrations in sentinel fish species permits an assessment of changing conditions, natural or anthropogenic impacts on upstream watersheds, and of potential regional influences on human and wildlife consumers of these fish species. Crevalle jack (Caranx hippos) and Gray snapper (Lutjanus griseus) are two common and widely distributed Florida estuarine fishes that are known to accumulate mercury. From 2006-2008, we assessed total mercury concentrations in these two species from 13 broad south Florida estuarine regions situated downstream of proposed Everglades restoration activities. Mean mercury concentrations in jacks and snappers from Florida Bay and Card/Barnes Sound estuaries adjacent to the Taylor Slough watershed were significantly higher than concentrations recorded in neighboring south Florida estuaries, and were as much as 2.0 and 2.7 times higher than concentrations measured in jacks and snappers, respectively, from central Florida Atlantic coast estuaries (i.e., Loxahatchee estuary, St. Lucie estuary, and Indian River Lagoon). Mean mercury concentrations in snappers from many southwest Florida Gulf coast estuaries (0.13—0.28 mg/kg) also exceeded mercury concentrations in snappers collected from neighboring offshore Gulf waters during the same time period (0.14 mg/kg in 2007-2008) and more recently (0.15 mg/kg in 2014-2015). These results provide a comprehensive landscape-scale baseline that can be used to identify mercury hotspots in south Florida, guide future research efforts in the region, and to monitor temporal and spatial changes in estuarine and coastal mercury concentrations as future Everglades restoration activities are implemented.

MP024 A Summary of the Scientific, Administrative and Legal Deficiencies in Florida's Statewide Mercury Total Maximum Daily Load Plan

L.E. Fink, Waterwise Consulting, LLC / Owner and Principal

The U.S. Environmental Protection Agency (EPA) protects the water quality of the nation's waters by reviewing and approving or rejecting and issuing a Total Maximum Daily Load (TMDL) for each water quality-impaired water body that cannot attain each of its duly promulgated Water Quality Standard (WQS) to protect aquatic life and human health after all point sources are in compliance with their technology-based effluent limits. The TMDL is the mass assimilation capacity under seasonally appropriate infrequent design conditions with an explicit or implicit ample margin of safety. The Waste Load Allocation is then divided fairly among all permitted point and nonpoint sources after subtracting the uncontrollable nonpoint source loads. For the WQS to be fully protective of human health, humans must be able to consume fish and shellfish from those waters at rates that do not present an unreasonable risk of diminution of the quantity or quality of life otherwise granted to each citizen by its creator. EPA calculated the methylmercury (MeHg) reference does (RfD) by dividing the lowest observable adverse effect level from an epidemiological study by an appropriate margin of safety. EPA ten translated the RfD into the equivalent total mercury (THg) concentration in fish flesh,by subtracting the national daily average MeHg background dose rate from the MeHg RfD multiplied by the national average woman's body weight and then divided that difference by the national average fish consumption rate multiplied by the trophic level

1, 2 and 3 diet weighted-average bioaccumulation factor for freshwater fish. When this is done using the higher Florida average fish consumption rates and marine fish THg levels, the THg WQC in fish flesh is 0. To avoid this conundrum, Florida adopted a probabilistic risk assessment approach with probability distributions functions for some but not all factors used by EPA in calculating the national MeHg as THg WQC. This resulted in an underestimate of health risks posed to the representative most susceptible fetus when its mother consumes fresh and salt water fish with Florida average THg levels during her pregnancy, let alone at subsistence rates from the most popular fisheries like the Everglades and Florida Bay, both contaminated above Florida's statewide 90th percentile cut-off. It is in this context that I summarize the scientific, administrative and legal deficiencies in Florida's Statewide Mercury TMDL Plan approved by EPA Region 4.

MP025 A contrast in mercury methylating populations and processes at high (U3 in WCA-2A) and low (DB-15 in WCA-3A) sulfate locations in the Everglades

H. Bae, Univ of Florida IFAS / Dept of Soil and Water Science; A. Ogram, Univ of Florida / IFAS / Soil and Water Sciences; F. Dierberg, DB Environmental, Inc.

Methylation of inorganic mercury is the first step leading to bioaccumulation and transfer of mercury across trophic levels. It is generally assumed that sulfate reduction is dominant process controlling mercury methylation in most environments; however, recent studies have shown that the microbial ecology of methylation may be much more complex than previously thought. In this study, we compared the dominant microbial groups responsible for mercury methylation in detritus (surficial floc) in areas of the Everglades with relatively high (U3) and low (DB-15) sulfate concentrations. Somewhat surprisingly, concentrations of methyl mercury (MeHg) were significantly higher in detritus at DB-15 than in U3. This observation is consistent with the relative concentrations of genes linked to mercury methylation (hcaAB), which were ten-fold greater at DB-15 than U3. In addition, genes characteristic of sulfate reducers (SRB) and syntrophs (dsrB) and methanogens (mcrA) were ten-fold higher in DB-15 than in U3 detritus. Note that concentrations of genes do not necessarily represent actual metabolic activities, but rather the potential for activity. Significantly, hgcAB sequences from DB-15 soil and periphyton were also dominated by methanogens; in contrast, most hgcAB sequences in U3 were associated with SRB. We suggest that mercury methylation in DB-15 is controlled in part by methanogens, and that the relatively high dsrB numbers in DB-15 represent syntrophs that also contribute to mercury methylation. This is consistent with the much higher ratios of the concentrations of dissolved organic carbon: sulfate, and lower pore water sulfide concentrations in DB-15 than in U3. The relatively low concentrations of MeHg at U3 may be the result of methylation by SRB, coupled with potentially higher rates of mercury demethylation at this site. Previous studies have demonstrated that demethylation rates are increased with increased sulfate concentrations in the Everglades (Marvin-DiPasquale and Oremland, 1998. ES&T. 32:2556-2563). In conclusion, controls on MeHg concentrations in the Everglades are complex and include methylation by different functional groups of microorganisms whose activities and physiologies are controlled by an array of environmental factors. At U3 and DB-15, the interplay between methanogens and SRB, and between the competing processes of mercury methylation and MeHg demethylation, may explain differences in MeHg concentrations in detritus.

MP026 Removal of Mercury from Surface Water by Constructed Wetlands in South Florida, USA

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Stormwater Treatment Areas (STAs) in south Florida are constructed wetlands designed to remove phosphorus from stormwater runoff originating from upstream agricultural areas, Lake Okeechobee discharge and other upstream land uses. Studies have shown that wetlands can be a significant site of mercury methylation. Concerns were being raised that attempts to reduce downstream eutrophication could inadvertently exacerbate the mercury problem known to be present in the Everglades. As a result, South Florida Water Management District implemented a mercury monitoring program which includes the quarterly collection of inflow and outflow water samples in each STA for analyses of total mercury (THg) and methylmercury (MeHg) during the first three years of operation. Mercury loads and load reductions were calculated using the annual average concentration and hydrological flow data for each STA. Results indicate that STAs displayed net reduction of THg and MeHg with an overall average of 54% for THg and 48% for MeHg. The highest reduction (80%) for THg is found in STA-3/4 and the lowest (7%) is found in Taylor Creek STA. The highest MeHg reduction is found in Taylor Creek STA (67%) and the lowest (19%) in STA-2. During the monitoring period, a total of 3,924 grams and 114 grams of THg and MeHg, respectively, were removed annually from STA inflows. Removal efficiency is thought to be related to mercury load, wetland biogeochemistry and hydrology. Significant insights can be gained by additional studies of the mechanisms of MeHg production and reduction in constructed wetlands.

Particle- or Pollen-Bound Pesticides in the Environment

MP027 Neonicotinoid and phenylpyrazole insecticides and their metabolites: Presence in WWTP effluents and surface water samples

M. Woudneh, R.D. Grace, M.C. Hamilton, J. Cosgrove, SGS AXYS

Neonicotonid and phenylpyrazole insecticides have received significant attention mainly due to their toxicity to aquatic and terrestrial insects. Current analytical methods for these analytes do not measure insecticide metabolites, which are also toxic, and do not include the concentration of synergists, whose presence magnify the toxicity several times, producing under estimated toxicity data for water samples. In the present work, we have developed isotope dilution LC-MS/MS method for the quantitative measurement of 8 neonicotinoids and 6 metabolites, as well as the phenylpyrazole fipronil and 4 of its degradation products. To help with better estimation of water toxicity data the method's analyte list also included three most commonly used synergists; piperonil butoxide, piperonil sulfoxide and MGK 264. The developed method was used for analysis of WWTP effluents and aqueous samples from agricultural areas. Distribution of the insecticides/metabolites dissolved in the aqueous phase and adsorbed to the solid phase was measured and their relative partitioning will be discussed. Ultimately these data contribute to our understanding of the fate and behaviour of some of the most widely used insecticides in the world.

MP028 Neonicotinoid Insecticides and Other Particle Bound Pesticides in the Atmosphere at Agricultural Impacted Sites

R.J. Raina-Fulton, Univ of Regina / Dept of Chemistry Biochemistry

Neonicotinoid insecticides partition between the gas and particle phase in the atmosphere and have a significant particle phase fraction. Fungicides including strobilurin fungicides also partition in the particle phase in the atmosphere. Neonicotinoids and a variety of fungicides have been used on a wide range of crops and particularly for neonicotinoid insecticides have varied application methods including foliar sprays, soil and seed treatments. This study investigates the seasonal atmospheric concentrations of neonicotinoids insecticides and selected fungicides in the particle phase in the atmosphere under field conditions within agricultural regions impacted by applications on varied crops with different schedules to give a real life seasonal trend on the occurrence of these insecticides and fungicides in the particle phase of the atmosphere. The air sampling is completed with a particle/vapour high-volume air sampler with a PM2.5 cyclone attached to the sampling head at most sampling sites. Study area includes Canadian sites within Okanagan Valley, Lower Fraser Valley, and Prairies and US sites within Yakima Valley, Okanogan County, and Prairies. In addition sampling took place at Glacier and Mt. Rainier National Park. These regions were also selected to allow for determination of long-range atmospheric transport events. The initial method development took place at sites in the Canadian Okanagan Valley where there are orchards and vineyards and extended into similar agricultural regions in Okanogan County and Yakima Valley of the United States. Detected neonicotinoids in the particle include imidaclopyrid, clothianidin, and acetamiprid with the highest concentration of 360.0 pg/m^3 for imidacloprid in 2013. Strobilurin fungicides detected included pyraclostrobin, trifloxystrobin, fluoxastrobin, and fluoxastrobin with pyrastrobin concentrations reaching 656 pg/m^3 in 2013. These analysis in 2013 did not include the full seasonal trends so new analysis of samples collected in 2014 or 2015 will be presented to provide a more complete picture of the seasonal variation. The LC/MS/MS method developed in 2015 included 7 neonicotinoid insecticides and 6 strobilurin fungicides in the particle phase and an updated method will be presented with consideration of inclusion of degradation products and other particle associated insecticides and fungicides. Data on other particle bound pesticides will also be presented which utilize GC/MS methods.

Plastic Debris: From the Continent to the Sea

MP029 Quantifying a source of microplastics: Shedding of synthetic microfibers from textiles

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Sources, occurrence and fate of microplastics in the environment are a subject of intense research, and they pose a potential threat to aquatic and marine organisms. Plastic fibers, presumably from textiles, have been indicated as a major source of this type of microplastic pollutant. These fibers have been identified in the majority of samples tested, ranging from freshwater to marine systems, surface and deep waters, sediment as well as biota from numerous trophic levels. These fibers are thought to be entering the oceans via washing machines and wastewater treatment plants, as well as house hold dust. In this study, the amount of microfibers shedding from synthetic textile fabrics was quantified for three different materials; acrylic, polyamide and polyester. All synthetic textile fabrics in the study were found to shed, but fleece and microfleece fabrics shed by far the greatest amount of fibers, up to 7360 fibers per m^2 in one wash. Tests showed a higher fiber loss when washing fabrics with detergent rather than without (as has been done in previous reports estimating fiber loss). A greater fiber loss was found among the fabrics that had been repolished to simulate wear, indicating that older garments might shed more as they age and become worn. However, the greatest number of fibers are lost during initial washing of new textile samples. The authors therefore propose that prewashing of all fabrics and garments at factory sites, followed by careful contaimnent of lost fibers at this pre-consumer stage, could be a means of reducing fiber loss to the environment. This study has also applied and optimized industrial standardized washing test, normally used in coloring tests.

MP030 Quantifying and characterizing plastic debris in a south Texas wastewater treatment system

J.L. Conkle, Texas A&M Univ Corpus Christi / Physical and Environmental Sciencees; E. Waddell, Texas A&M Univ Corpus Christi

It is often stated the wastewater treatment plant (WWTP) effluent is one of the major ways in which plastic enters the environment. While this may be true, only a handful of studies have actually examined WWTPs to quantify and examine the characteristics of plastics from this source. In those studies, the WWTPs removed the majority of plastic particles. However, there has been less work on plastic fibers in these systems. In this study, the influent, effluent and biosolids of a wastewater treatment plant with a capacity of 8 million gallons per day was sampled. The objectives of this study were to quantify and characterize plastic entering the WWTP and assess its fate. To accomplish this, samples were taken bi-weekly at a Corpus Christi, TX WWTP for 3 months. Samples were then sieved for plastic particles and fibers at 50 and 300 mm. This research is ongoing, but the expectation is that both plastic particles and fibers will be found in the system, with fibers outnumbered the particles.

MP031 New Methods for Recovery of Microplastics from Digestive and Respiratory Tissue

E.N. Waddell, Texas A&M Univ-Corpus Christi / Environmental Science

Plastic materials are nearly ubiquitous throughout the marine environment, where they negatively impact some marine organisms. Once in the environment they can be consumed actively/deliberately or passively (by filter feeding or when associated with other food materials) in addition to becoming entangled in gills. This uptake of microplastics can result in the bioaccumulation of plastics, necessitating research to quantify microplastics in lower trophic level marine organisms. Extraction and identification of microplastics can be challenging due to their small size and the complexity of the organismal structures in which they accumulate. Some existing extraction methods use concentrated nitric acid and hypochlorous acid to breakdown organismal tissue. However, these chemicals rapidly dissolve nylon, which is commonly used for fishing line, fabrics, ropes, etc. and therefore prevalent in the environment. Due to this presence, it is important to utilize methods that account for nylon when quantifying plastic loads in marine organisms. To quantify microplastic loads in blue crabs (Callinectes sapidus), a new method has been developed where the soft tissues of the digestive system are dissolved using 40% hydrogen peroxide, followed by hot filtration, while microplastics associated with the the non-dissolvable gills are removed using sonication and density separation.

MP032 Microscopic anthropogenic litter in terrestrial birds from Shanghai, China: Not only plastics but also natural fibers

S. Zhao, D. Li, East China Normal Univ

The level of contamination by microscopic anthropogenic litter (0.5–5 mm) in terrestrial ecosystems is not well understood. After chemical digestion in 10% KOH, microscopic anthropogenic litter from the gastrointestinal tracts of 17 terrestrial birds was identified and categorized under a stereomicroscope based on its physical properties and melting tests. In total, 364 items from 16 birds were identified as microscopic anthropogenic litter, ranging in size from 0.5 to 8.5 mm. No relationship between plastic load and body condition was found. Natural fibers, plasticfibers and fragmented plastics represented, respectively, 37.4% (136 items), 54.9% (200 items) and 7.7% (28 items) of total litter items. Small sample sizes limited our ability to draw strong conclusions about the metabolism of naturalfibers, but the decline in the proportion of naturalfibers from the esophagus to stomach to intestine suggested that they may be digestible. Particles smaller than 5 mm represented more than 90% of the total number of pollutant items. Particles with colors in the mid-tones andfibrous shapes were overwhelmingly common particles. The results reflect pollution by microscopic anthropogenic litter in the terrestrial ecosystem of the study area. Microscopic naturalfibers, which may disperse and adsorb chemical pollutants differently from microplastic and may pose an even greater risk, are in urgent need of further research.

MP033 Consequences of environmentally relevant concentrations of microplastic with and without PCBs in a freshwater food chain

C.M. Rochman, UC Davis / Ecology and Evolutionary Biology; S. Teh, Univ of California – Davis; S. Serrato, UC Davis; E.J. Reiner, Ontario Ministry of Environment / Laboratory Services Branch; M. Robson, Brock Univ / Chemistry

Plastic debris has now been documented in several freshwater habitats and animals. Much of this material is microplastic (< 5 mm in size) and is associated with a cocktail of chemicals added during manufacturing that are persistent, bioaccumulative and toxic (PBT). This combination of microplastic and PBTs are likely ingested by freshwater organisms at various trophic levels in nature. Ingestion of microplastic provides another pathway for sorbed chemicals and those already associated with microplastic to transfer to animals with uncertain health effects. Furthermore, there is little information on the extent to which ingesting microplastics may enhance biomagnification in aquatic foodwebs. To provide a better understanding of how microplastic affects chemical transfer and how different types of microplastic affects the health of freshwater organisms we designed an experiment using Asian clams (Corbicula fluminea) and white sturgeon (Acipenser transmontanus). We quantified the pathway for microplastics to transfer sorbed PCBs to prey, measured the chemical transfer of PCBs from prey to a predator and determined how the ingestion of various polymers, with and without PCBs, affects organismal health. Asian clams were exposed for 30 days to separate treatments of microplastic (polyethylene terephthalate, polyethylene, polyvinyl chloride and polystyrene) with and without sorbed PCBs. Next, diets were formulated using purified ingredients and clams from the first exposure and fed to their predators (sturgeon) for 30 days. Chemical analyses using GC/MS were used to analyze concentrations of PCB congeners from microsplastic in animal tissues, allowing us to measure any bioaccumulation and biomagnification. Several toxicological assays, including histophathology and immunohistochemistry were used to measure effects related to exposure to various types of microplastic with and without PCBs. Results of these experiments will be presented.

History and Role of SETAC in the Advancements in Environmental Chemistry and Aquatic Toxicology

MP034 Linking academic training to the 'real world': Adventures in an internship with industry

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We as scientists interpret data from precise observations to inform logical hypotheses and experienced choices, yet when we graduate we are meant to decide upon a career path in one of several fields including academia, industry, and government, without prior experience to inform our decisions. Graduate studies are inherently niche experiences as students are experts in a specific realm of toxicology, generally in the context of academia. In the field of toxicology, which can be viewed as an applied science, it is particularly important to create well informed and experienced individuals who understand the context of their science in the 'real-world'. One method with which to gain experience in different sectors is through internships. For example, some institutions including the Toxicology Centre at the University of Saskatchewan (U of S) and the Ecotoxicology Group at Shell have recently made strides to improve the breadth of experience of graduate students. Students at the U of S who participate in the NSERC funded Human and Ecological Risk Assessment (HERA) program are mandated to complete internships within government, academia, or industry, while Shell has recently initiated a graduate level internship program. These programs help students to understand the context of their science and develop a more informed individual who is better prepared for the challenges following completion of their degree. These programs are unique in that they allow students to apply concepts learned in their studies to real scenarios, effectively demonstrating the value of their training. Further, experience gained from an internship can help to identify gaps in marketable skills and encourage greater thought into ideas of environmental significance and applicability of data, which are not always present in an academic setting. The industry sponsor benefits from having enhanced academic connections, insight into current training methods, a pipeline of talent, and a fresh perspective on their issues. Internship programs such as those initiated at the U of S and the Ecotoxicology group at Shell are unique opportunities for students to gain experience in a diversity of fields and were formed in the tripartite spirit of the Society for Environmental Toxicology and Chemistry. In this presentation we will outline some of the projects and educational experiences of the first generation of Shell interns and discuss the benefits for both the student and the industrial sponsor.

Exposure, Effects and Fate of New Organic Contaminants in Aquatic Ecosystems

MP035 Polyhalogenated Carbazoles in Aquatic Sediments from the United States and China

Y. Wu, D. Chen, Southern Illinois Univ Carbondale / Cooperative Wildlife Research Laboratory and Dept of Zoology; R. Sutton, San Francisco Estuary Inst; K. Xu, Louisiana State Univ / Dept of Oceanography and Coastal Sciences; J. Moore, US Fish and Wildlife Service / Interior; K. Grasman, Calvin College / Dept of Biology; Y. Qiu, Tongi Univ

The present study reports the discovery of a suite of polyhalogenated carbazoles (PHCZs) in aquatic sediments collected from five watersheds located in United States and China, including the Saginaw River system (Michigan, USA), Gulf of Mexico (USA), San Francisco Bay (USA), Lake Tai (China), and Lake Dianshan (China). While halogenated carbazoles have recently been reported in sediments from the Great Lakes of North America and a few other aquatic systems in Europe, data on their occurrence remain overall limited worldwide. In this study, 11 halogenated carbazoles, including 3-chloro, 3,6-dichloro-, 1,3,6,8-tetrachloro-, 2,3,6,7-tetrachloro-, 3-bromo-, 2,7-dibromo-, 3,6-dibromo-, 1,3,6-tribromo-, 1,3,6,8-tetrabromo-, 1-bromo-3,6-dichloro-, and 1,8-dibromo-3,6-dichloro-carbazole were screened. Halogenated carbazoles were detected in 98.7% of the sediment samples, with concentrations ranging from below method limits of quantification to 51.5 ng/g dry weight. In most of these sediment samples, PHCZ concentrations exceeded those of polybrominated flame retardants (PBDEs). The latter group of chemicals has been demonstrated to be persistent and globally distributed. In general, 3,6-dichlorocarbazole was consistently the dominant congener, followed by 3-chlorocarbazole, in the studied watersheds, except for the Lake Tai sediments, where. 3,6-dibromocarbzole rivaled 3,6-dichlorocarbazole in concentrations. Additionally, 1,3,6-tribromo-, 1,3,6,8-tetrabromo, 1-bromo-3,6-dichloro-, and 1,8-dibromo-3,6-dichloro-carbazole were also frequently detected. These findings demonstrated that polyhalogenated carbazoles are widely distributed in marine and freshwater systems in the USA and China. They may represent another group of persistent organic pollutants with global distribution and dioxin-like effects. Future investigations are needed to elucidate their sources and fate, as well as ecological risks, in global aquatic environments.

MP036 Chemicals of Emerging Concern (CECs) in the Saginaw River System

Y. Wu, H. Tan, Southern Illinois Univ Carbondale / Cooperative Wildlife Research Laboratory and Dept of Zoology; J. Moore, US Fish and Wildlife Service / Interior; K. Grasman, Calvin College / Dept of Biology; L.L. Williams, US Fish & Wildlife Service; D. Chen, Southern Illinois Univ Carbondale / Cooperative Wildlife Research Laboratory and Dept of Zoology

The present study investigated a variety of chemicals of emerging concern (CECs), including flame retardants and polyhalogenated carbazoles (PHCZs), in the Saginaw River System (Michigan, USA) and the Saginaw Bay of Lake Huron. Surface water samples were collected from 14 representative sites in the watershed in the spring and summer of 2014 and 2015, and surficial sediments were collected from the same sites in 2014. After sample cleanup by gel permeation chromatography and solid phase extraction, target compounds were quantitatively determined on gas chromatography coupled to a single quadrupole mass spectrometer. In sediments, concentrations of organophosphate flame retardants (OPFRs) ranged from 2 to 151 ng/g dry weight (dw), significantly greater than the levels (0.11 – 42 ng/g dw) of polybrominated flame retardants (PBDEs). Additional flame retardants, such as bis(2-ethylhexyl)-3,4,5,6-tetrabromophthalate (BEH-TBP), bis(2,4,6-tribromophenoxy)ethane (BTBPE), tetrabromo-o-chlorotoluene (TBCT), and 1,2,5,6-tetrabromocyclooctane (TBCO) were also frequently detected in sediments. The total concentrations of non-PBDE, alternative flame retardants (excluding OPFRs) ranged from 0.1 to 22.9 ng/g dw. In surface water, concentrations of OPFRs (16.6 – 418 ng/L) far exceeded those of PBDEs (non-detection to 1.0 ng/L) and other flame retardants (generally no detection). In addition to flame retardants, PHCZs were also widely distributed in the watershed, with concentrations in sediments of 1.4 – 68.6 ng/g dw. PHCZs are an emerging group of persistent organic pollutants possessing dioxin-like effects. Overall, our findings suggest that emerging flame retardants are widely distributed in the Saginaw River system. Use of some of them has been increasing as replacements for PBDEs in a variety of applications. Therefore, environmental monitoring is needed to track the concentrations of these flame retardants and other CECs over time in the Saginaw River watershed and assess whether they may be posing risks to biota.

MP037 Source and Risk Assessment of Persistent Organic Pollutants (POPs) in Water and Sediments from the Bosten Lake, Northwest of China

W. Chen, Lancaster Univ / School of Environmental Studies; F. Peng, S. Qi, C. Qu, X. Xing, China Univ of Geosciences / School of Environmental Studies

The Bosten Lake is the largest inland freshwater lake in China and among the most important water sources in the arid region of Northwest China. To study the distribution and source of the pollutants in this region is essential for protection of the fragile ecosystem and further assessment of potential risk to ecosystem and human health. Water and sediments samples were collected from the Bosten Lake and analysed for persistent organic pollutants (POPs). Our previous study (Chen et al, 2011) showed that the all the organochlorine pesticides (OCPs) except o,p'-DDT were detected in sediments from the Peacock River which supplied by the Bosten Lake, indicating that Bosten Lake and/or agricultural tailing water returned directly into the Peacock River could be a possible source of OCPs. The results from the Bosten Lake showed that the OCPs were also widely detected in both water and sediments. The distribution characteristics of OCPs are impacted by the human activities around. The input accompanied with water and suspended particulate matters from the Kaidu River, atmospheric transport and human activities could be the main sources of the POPs in the Bosten Lake. As the important source of the water supply, the potential risk to the ecosystem and human health for POPs couldn't be disregard in the Bosten Lake region.

MP038 Chronic and multigenerational toxicity evaluation of the flame retardant tris (2-butoxyethyl) phosphate (TBOEP) using Daphnia magna

M. Lépine, M. Giraudo, M. Houde, Environment and Climate Change Canada; M. Houde, Environment and Climate Change Canada / St Lawrence Center

Tris (2-butoxyethyl) phosphate (TBOEP) is an organophosphorus flame retardant of high production volume used in floor waxes and as plasticizers in a wide range of applications. The use of TBOEP containing products has resulted in its release and ubiquitous occurrence in the aquatic environment. In this study, three life-history endpoints (survival, growth, and reproduction) were evaluated in Daphnia magna to investigate sublethal effects of TBOEP. Chronic exposure (21 days) to an environmentally relevant concentration of TBOEP (10 µg/L) was carried out over three successive generations. Exposures to TBEOP did not impact survival and reproduction but altered the growth of zooplankton. The mean number of molts was found to be significantly lower in D. magna exposed to TBEOP compared to controls for a given generation, however no differences were observed among generations. Results also indicated potential negative effects over generations on the size of exposed organisms. Complementary epigenetic (i.e., DNA methylation) and transcriptomic analyses will help to better understand these results and to study the impact of TBOEP on the molecular level. Results highlight the relevance of chronic and multigenerational biological evaluation for environmental risk assessments for emerging chemicals.

MP039 Transcriptomic response of Daphnia magna exposed to benzotriazoles: endocrine-disrupting potential and molting effects

M. Giraudo, M. Douville, Environment and Climate Change Canada; G. Cottin, Universite Paris Descartes; M. Houde, Environment and Climate Change Canada

Benzotriazoles (BZTs) are ubiquitous aquatic contaminants used in a wide range of industrial and domestic applications from aircraft deicers to dishwasher tablets. Acute toxicity has been reported in aquatic organisms for some of the BZTs but their mode of action remains unknown. The objectives of this study were to evaluate the transcriptomic response of D. magna exposed to sublethal doses of 1H-benzotriazole (BTR), 5-methyl-1H-benzotriazole (5MeBTR) and 5-chloro-1H-benzotriazole (5ClBTR) using RNA-sequencing and quantitative real-time PCR. Cellular and life-history endpoints (survival, reproduction, growth) were also investigated. Significant effects on the molting frequency were observed after 21-d exposure to 5MeBTR and 5ClBTR. Molting in cladocerans is closely intertwined with growth and reproduction and is actively controlled by ecdysteroid hormones. No effects on molting frequency were observed for BTR but RNA-seq results indicated that this BZT induced the up-regulation of genes coding for cuticular proteins which could have compensated the molting disruption. Complementary short-term temporal analysis (4- and 8-d exposure) of the transcription of genes related to molting and hormone-mediated processes indicated that both BTR and 5ClBTR induced an over-transcription of cyp18a1 with time, which is a negative regulator of the ecdysteroid receptors EcR and USP. Cyp18a1 induction could have resulted in the observed reduction in molt frequency in 5ClBTR-exposed animals, further explained by the concomitant decrease of cuticle genes transcription. On the other hand, the increased number of molts measured in response to 5MeBTR could have been induced by the decreased transcription of cyp18a1 and ecr/usp and the over-transcription of cuticular genes measured in exposed-organism. Measurement of the activity of the molting enzyme chitinase is presently being conducted in order to better understand the effects of BZT on the growth of D. magna. Overall, results suggested that molting effects observed at the physiological level could be linked to endocrine regulation impacts of BZTs at the molecular level.

MP040 Oxygen-functional Groups & Ready Biodegradability of Flavor and Fragrance Molecules

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For the environmental fate of organic chemicals, their biodegradation potential is an essential intrinsic property. A key factor effecting biodegradability of chemicals might be their functional groups and their molecular structures. Therefore, the aim of this work was to investigate the influence of oxygen functional groups and the molecular structure on the biodegradation potential of a numerous flavor and fragrance molecules. The results showed that different oxygen functional groups contained in the molecules have variably effects on the biodegradation potential of the chemicals. For instance, ester-, formate-, hydroxyl- and carboxylic groups have a significant enhancing effect, whereas ether-, acetale-, epoxide groups show remarkable inhibiting effects on the biodegradation potential of the molecule. Furthermore, for some functional groups such as ketones and aldehydes, the biodegradation potential depends on their position in the molecule (e.g. direct or indirect binding to a ring system). Based on these results it might be possible to modify the biodegradation potential of chemicals by introducing certain oxygen function groups into the molecules or by modifying their molecular structures. These outcomes provide a perspective for the development of environment friendly "green chemicals".

MP041 Pharmaceutical and personal care product residues in wastewater treatment plant samples from Kentucky and Georgia, USA

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The occurrence of pharmaceutical and personal care product (PPCP) residues in the environment has received considerable attention in the recent years as these compounds have been implicated for negative effect on biota and the ecosystem. PPCPs are considered emerging environmental pollutants, and have been detected in groundwater, surface water and municipal wastewater, fish and biosolids. The present study was aimed at determining baseline levels of pharmaceutical chemicals such as macrolide antibiotics, carbamazepine, personal care products namely triclosan, polycyclic musks in influent and effluent samples from wastewater treatment plants in Murray, Kentucky and Savannah, Georgia, USA. Influent, effluent, return activated sludge, pre-chlorination, post-chlorination, upstream from the WWTP, and downstream samples were analyzed following the approved procedures. Detectable levels of pharmaceutical chemicals as well as personal care products were found in most of the samples analyzed. In general, influents contained higher concentrations of the analytes than the effluents. Presence of azithromycin, carbamazepine, triclosan and polycyclic musks in the effluents indicated that these compounds are persistent and are not degraded/or lost during the wastewater treatment processes and contaminate the receiving water bodies such as streams and rivers.

MP042 Temporal trends of volatile methylsiloxanes in Tokyo Bay sediment core, Japan

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Methylsiloxanes have been widely used in personal-care and house hold products because methylsiloxanes have low surface tension, high thermal and chemical stabilities. However, a part of volatile methylsiloxanes (VMSs) have recently been identified as priority chemicals for environmental risk assessment due to their persistence in the environment and bioaccumulative potency. To reveal temporal trends of VMS accumulation into the aquatic environment, in this study, we investigated the vertical concentrations of cyclic and linear VMSs (7 VMSs including D3, D4, D5, D6, L3, L4, and L5) in a sediment core collected from Tokyo Bay, Japan. The sediment core was collected in July 2013 from the inner bay (N35°35'00", E139°55'00"), using an acrylic tube. The sediment core (86 cm length), sliced at 2-cm increment was analyzed. The time interval of each slice were estimated based on sedimentation rate (1 cm/y, the data from previous study) and sedimentation depth. Cyclic VMSs were found throughout the sediment core upper 38 cm sections, at ΣVMS ranging from 10 to 700 ng/g dry wt. The highest concentration of ΣVMS was found in the 10-12 cm section of the core. The concentrations of ΣVMS increased with decreasing depth of upto 10-12 cm, corresponding to the year 2001-2003, then slightly decreased (almost constant) upto the surface layer to the year 2013 (630 ng/g dry wt). Interestingly, the vertical profiles of D4 concentrations were different from other cyclic VMSs; the highest concentration of D4 was found in the 14-16 cm section (the mid-1990s), then D4 concentrations decreased upto the surface layer. In general, the vertical profiles of organic compounds in a sediment core reflect production and usage trends and industrial activities. Replacement D4 to D5 in personal-care products such as shampoo and cosmetics in the mid-1990s appear to have been effective in reducing the input of D4 to the aquatic environment, whereas ΣVMS concentrations were found to be almost constant in last decade. The annual flux of VMSs into the Tokyo Bay sediments in 2004-2013 (in average) was calculated to be 2.4 ng/cm^2 for D4, 90 ng/cm^2 for D5, 16 ng/cm^2 for D6, and 110 ng/cm^2 for ΣVMS. Based on the surface area of Tokyo Bay (922 km^2), annual deposition of ΣVMS into Tokyo Bay sediment were roughly estimated at 1000 kg.

MP043 Glyphosate-induced toxicity in African catfish (Clarias gariepinus, Burchell) following a sub-lethal exposure

M.M. Salman, O.T. Aladesanmi, Obafemi Awolowo Univ / Inst of Ecology and Environmental studies

Glyphosate is a non-selective herbicide which has been severally used to control weeds. The present study aimed to examine the cytotoxic and haematological effects of glyphosate on Clarias gariepinus. Sixty fishes were exposed to acute concentrations of 54, 108, 162, 216 and 270 mg/l of glyphosate with two replicates and two controls for 96 hrs. Thereafter, the median lethal concentration (LC_{50}) was calculated using probit analysis. Subsequently, 21 days bioassay was employed where one hundred and eight fishes were exposed to sub-lethal concentrations of 0, 9, 18, 27, 36 and 45 mg/l in two replicates. Blood samples were further collected through caudal severance and analysed using an auto-analyzer machine. In addition, the chromosome spreads were prepared and the photomicrographs were taken. The dividing cells were counted with respect to the total number of cells in the slides in order to determine the mitotic index. Behavioural responses such as vertical swimming, flaring of operculae and gasping were observed. The LC_{50} obtained was 167.76 mg/l while a dose-dependent increase in chromosomal stickiness and decrease in mitotic index was observed. Similarly, the erythrocyte, leucocyte, haemoglobin, and haematocrit decreased dose-dependently while the mean corpuscular volume and mean corpuscular haemoglobin increased dose-dependently. Fluctuating pattern was however recorded for the mean corpuscular haemoglobin concentration. Behavioural responses such as surface swimming and mouth opening denotes respiratory impairment in the fishes. Also, the reduced erythrocyte, haematocrit and haemoglobin recorded denote anaemic condition in exposed fishes. The decreased leucocyte predisposes the fishes to high risk of disease infection while the sticky chromosome depicts respiratory impairment. In addition, the dose-dependent decrease in mitotic index indicates impairment of cell division in the exposed fishes. Considering the abnormal effects revealed, glyphosate herbicide is toxic to Clarias gariepinus. Therefore, its use on/near aquatic environment should be highly restricted, if not prohibited.

MP044 Developmental Toxicity and Mechanistic Investigations of Nitrated and Heterocyclic PAHs

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Nitrated polycyclic aromatic hydrocarbons (NPAHs) and heterocyclic PAHs (HPAHs) are recognized environmental pollutants, with sources including petroleum-based products, combustion, in particular diesel, and formation by atmospheric reactions with unsubstituted PAHs. However, the health risks of NPAHs and HPAHs to humans and environmental systems are not well-studied. The developmental zebrafish (Danio rerio) model was used to evaluate the toxicity of a structurally diverse set of 27 NPAHs and 10 HPAHs. Their individual activities towards the aryl hydrocarbon receptor (AHR), including the role of the AHR in observed toxicity, and genetic markers of oxidative stress and cardiac toxicity were evaluated. All 25 commercially-available NPAHs were assessed, as well as two NPAHs synthesized in-house, which are not commercially available, in addition to 10 commercially available HPAHs. Zebrafish embryos were exposed from 6 to 120 hours post fertilization (hpf) to a dilution series of individual compounds and evaluated for 22 developmental endpoints. The potential role of AHR was determined via CYP1A immunohistochemistry (IHC), as well as a GFP/CYP1A reporter zebrafish line. All compounds were screened computationally through molecular docking into previously developed AHR models of zebrafish isoforms 1A, 1B, and 2. The docking scores, representing AHR binding and activation, predicted the in vivo CYP1A response with a success rate of 69%. Some compounds did not induce observable developmental toxic responses, while others produced statistically significant concentration-dependent toxicity. The tested compounds also exhibited a range of AHR binding

and CYP1A induction patterns, including CYP1A expression in the liver, vasculature, skin, and yolk, which we determined to be due to distinct isoforms of the AHR using morpholino oligonucleotide knockdown. Furthermore, we investigated mRNA expression of oxidative and cardiac stress genes at 48 and 120 hpf, investigating potential mechanisms-of-action for NPAHs. We also compared the developmental toxicity of two amino PAHs (metabolites of NPAHs) to the corresponding NPAHs, and observed equal or increased toxicity in the amino PAHs compared to the corresponding NPAHs. This indicates the need for further experimental and in silico studies of NPAHs and HPAHs as environmental contaminants with the potential for adverse human health effects, including further investigation into the mechanisms-of-action and metabolism.

MP045 Contaminants of Emerging Concern in Herring Gull and Caspian Tern Eggs From United States Colony Sites in the Great Lakes of North America

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Numerous chemicals have been designed, synthesized and widely used to various manufactured materials such as plastics, foams, textiles, furniture, paper coating and many others materials. These chemicals include various organic flame retardants (OFRs) and per- and polyfluoroalkyl substances (PFASs). Both FRs and PFASs have been reported in the environment in fish, wildlife and humans, and including in birds from the Laurentian Great Lakes of North America with a focus on the eggs of herring gulls (Larus argentatus). The Canada-U.S. International Joint Commission (IJC) renewed the U.S.-Canada Great Lakes Water Quality Agreement (GLWQA) on September 2012, which specifically calls for the identification and monitoring of chemicals of emerging (environmental) concern (CECs) in various matrices, i.e. animal species and especially in fish-eating birds. However, other than the herring gull (which is not a totally aquatic fish consumer), there is presently limited information on CECs in other bird species such as the purely fish-eating Caspian tern (Hydroprogne caspia). The Caspian tern is also currently a state-threatened species in Michigan. In the present study, a broad suit of CECs, including 26 polybrominated diphenyl ethers (PBDEs), 23 non-PBDEs halogenated FRs (NPHFRs), 16 organophosphate esters (OPEs), 4 perfluorinated sulfonates (PFSAs), 13 perfluorinated carboxylic acids (PFCAs) and 5 emerging perfluoroalkyl acids (PFAAs) or precursors, were determined and compared in 30 individual Caspian tern eggs and 10 herring gull eggs collected in 2013 and 2014 from U.S. / Michigan nesting sites on Two Tree Island (St, Marys River), Charity Reef (Saginaw Bay) and Channel-Shelter Island (a Confined Disposal Facility in Saginaw Bay). The general order of concentration levels in Caspian tern eggs was as follows: ΣPFSA (mean: 793 ng/g wet weight (ww); range:116-4690 ng/g ww) > ΣPFCAs (131; 30.4-506 ng/g ww) ≈ ΣPBDEs (86.7; 32.4-189 ng/g ww) >> ΣNPHFRs (0.67; ND-4.3 ng/g ww) ≈ ΣOPEs (0.46; ND-2.89 ng/g ww). Compared to gull eggs, tern eggs contained significantly lower concentrations of ΣPBDE and ΣNPHFR, but with up to 10 times greater mean concentrations of ΣPFSAs and ΣPFCAs. This indicates that terns are exposed to and bioaccumulate greater amounts of ΣPFSAs and ΣPFCAs than gulls as reflected in their respective egg concentrations, which is likely due to differences in the respective dietary consumption of these two species.

MP046 Temporal and spatial behavior of pharmaceuticals in Narragansett Bay, Rhode Island, United States

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The behavior of active pharmaceutical ingredients (APIs) in urban estuaries is not well understood. In this study, 15 high volume usage APIs were measured over a one year period throughout Narragansett Bay, RI, USA to determine factors controlling their concentrations and distribution. Dissolved APIs ranged in concentration from not detected to 310 ng/L, with numerous APIs present at all sites and sampling periods. Eight APIs were present in suspended particulate material, ranging in concentration from < 1 ng/g to 44 ng/g. Partitioning coefficients (K_ds) were determined for APIs present in both the dissolved and particulate phases, with their range and variability remaining relatively constant during the study. Organic carbon normalization reduced the observed variability of several APIs to a small extent; however, other factors appear to play a role in controlling partitioning behavior. The continuous discharge of wastewater treatment plant effluents into upper Narragansett Bay resulted in sustained levels of APIs, resulting in a zone of "pseudo-persistence". For most of the APIs, there was a strong relationship with salinity, indicating conservative behavior within the estuary. Short flushing times in Narragansett Bay coupled with APIs present primarily in the dissolved phase suggests that most APIs will be diluted and transported out of the estuary, with only small amounts of several compounds removed to and sequestered in sediments. This study identifies factors controlling the temporal and spatial dynamics of dissolved and particulate APIs, whose partitioning behaviors provide an increased understanding of their fate, including bioavailability, in an urban estuary.

MP047 Effect of substituted phenylamine antioxidants on three life stages of freshwater mussel Lampsilis siliquoidea

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Substituted phenylamine antioxidants (SPAs) are produced in relatively large volumes and incorporated into a variety of consumer products, e.g., polymers, lubricants, dyes, and adhesives. Based on their physical and chemical properties, SPAs could be potentially persistent, bioaccumulative and/or toxic, consequently, the Government of Canada is assessing this class of compounds under the Chemicals Management Plan for potential risks to the environment. The current study assessed the toxicity of four SPAs, chosen from a list of twelve, (diphenylamine (SPA 2), N-phenyl-1-naphthylamine (SPA 6), N-(1,3-dimethylbutyl)-N'-phenyl-1,4-phenylenediamine (SPA 8), and 4,4'-methylene-bis[N-sec-butylaniline] (SPA 12)) to three life stages of the freshwater mussel, Lampsilis siliquoidea. Acute sensitivity was determined by exposing the larval stage of mussels (glochidia) to SPAs for 48 h in water. A negative relationship between the toxicity of SPAs and their solubility in water was observed. The 48-h EC50s for glochidia viability using measured concentrations were 5951, 606, 439, and 258 µg/L for SPA 2, 6, 8, and 10, respectively. Juvenile (~ 1 cm in length) and adult Lampsilis siliquoidea were exposed to sediments spiked with individual SPAs for 28 days in aerated (static) vessels with a 3.5:1 water-to-sediment ratio. Mortality was assessed along with rate of feeding of juvenile mussels. In addition, reactive oxygen species, total glutathione, and lipid peroxidation in gill, digestive gland, and gonad of SPA-exposed adult mussels were measured to determine whether exposure to SPAs caused oxidative stress; bioaccumulation was also assessed in these tissues. Preliminary data analysis based on nominal concentrations showed that LC/EC50 values for the juvenile and adult mussels were >100 µg/g sediment dry weight for all four SPAs examined. Analysis of tissue and sediment is ongoing; calculation of biota-sediment accumulation factors will provide information on the bioavailability of SPAs to freshwater mussels. The results of this study will support environmental risk assessment activities to determine if SPAs could impact freshwater ecosystems.

Everglades and Wetlands Science Part 1: Ecology and Contaminants in Ever-Changing Ecosystems

MP048 Geospatial analysis of Perfluoroalkyl Acids in American Alligators (Alligator mississippiensis) in Florida and South Carolina

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This study aimed to geospatially assess Perfluoroalkyl acid (PFAA) plasma levels in the American alligator (Alligator mississippiensis) both across Florida and South Carolina (n = 125) and specifically at Merritt Island National Wildlife Refuge (MINWR) at Kennedy Space Center in Titusville, FL (n = 229). We investigate potential seasonal and spatial trends. This study provides a solid baseline for PFAA levels in a wetlands keystone species within the southeastern United States, and develops a more thorough understanding of crocodillian PFAA sex-based differences on a regional and local scale as well as PFAAs and snout to vent length (SVL) relationships.

MP049 The Combined Effects of Coal Combustion Waste and Pond Drying Regime on the Southern Toad (Anaxyrus terrestris)

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Energy production is one of the largest drivers of environmental change. Despite shifting towards an energy security model that embraces diversity in energy sources, coal still represents the single largest source of energy in the United States. The combustion of coal for energy results in the production of large amounts of solid waste in the form of coal combustion waste (CCW), which contains a number of potentially toxic trace elements. Nearly half of CCWs produced are stored in the environment, in landfills and surface retention basins. While these practices limit the amount of area impacted by this waste, they can create highly contaminated localized habitats where wildlife can come in direct contact with the CCW. Retention ponds that contain CCWs mixed with water often fail resulting in the surrounding landscape receiving large volumes of waste. CCWs have lethal and sub-lethal effects on a variety of aquatic life, including amphibians; however the influence of wetland drying regime on CCW toxicity has not yet been explored. Wetland drying can influence CCW toxicity by increasing the levels of trace elements and/or by stressing amphibian larvae that must accelerate development. We used simulated ponds (mesocosms) to examine how wetland drying regime and the presence of CCWs in sediments separately and interactively affect the growth, development, and survival of the southern toad (Anaxyrus terrestris). The presence of CCW sediments led to reduced mass and snout-vent-length at metamorphosis ($p < 0.05$), but did not delay development. We found no effect of drying regime on development or size at metamorphosis and no interaction between sediment type (with or without CCW) and drying regime. We used a Cox proportional hazard model, with mesocosm ID as a clustering term, to examine the effects of the treatments on probability of metamorphosis and found no consistent effects of the applied treatments. These data suggest that for southern toads, the effects of CCWs are consistent regardless of drying regime. We will be following up with analyses of initial size distributions of larvae for each tank, actual concentrations of trace elements in each tank over time, and body burdens acquired by individuals across tanks to attempt to better explain the among tank variation we observed in the study.

Advancing -Omics into Regulatory Frameworks: Case Studies and Perspectives

MP050 Bringing the fathead minnow (Pimephales promelas) into the genomic era

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The fathead minnow (Pimephales promelas)is a well-established ecotoxicological model organism that has been widely used for regulatory ecotoxicity testing and research for over a half century. Throughout this time, a lot of knowledge has been gained about the fathead minnow's biological responses to various xenobiotics. However, despite its importance as a model organism, the fathead minnow still has few publicly available gene sequences. Recently, Burns et al. (2015; Environ. Toxicol. Chem. 35:212) described the sequencing and de-novo assembly of the fathead minnow genome. Two draft genome assemblies are now publicly available on the GenBank database. However, on their own the draft assemblies remain of limited use to researchers who are primarily interested in the functional units of the genome, i.e. the genes. In the present study, an annotation pipeline, consisting of gene prediction, evidence alignment, and data synthesis, was applied to the fathead minnow SOAPdenovo assembly. Ab initio gene prediction was performed using AUGUSTUS, which provided a starting point of 43,345 gene predictions. Fathead minnow Expressed Sequence Tags (ESTs) and zebrafish protein-coding sequences (CDSs) were then aligned to the assembly using the corresponding spliced alignment methods of the program Exonerate. Of the over 240,000 EST alignments, 73% were successfully aligned with 90% or greater sequence identity and query coverage. Similarly, 39% of nearly 45,000 zebrafish coding sequences were successfully aligned with 70% or greater sequence identity and 50% or greater query coverage. The alignments and gene predictions were then passed into the program MAKER, which combined the weighted evidence from various sources into individual gene structures. Together, these data will provide a valuable annotated genome resource to the community and lay a foundation for future studies which make use of and build upon fathead minnow molecular sequence information. The contents of this abstract neither constitute nor necessarily reflect official USEPA policy.

MP051 Examining the effects of endocrine disruption on innate immunity in Rana (Lithobates) catesbeiana tadpoles

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Thyroid hormone (TH) facilitates a number of crucial processes in vertebrates, including growth, metabolism, and development. In frog tadpoles, TH is responsible for the metamorphosis of aquatic larvae into terrestrial frogs. During this process, the body of the animal undergoes extensive internal and external changes in preparation for a terrestrial life. The innate immune system, in particular, is significantly altered by TH modulation during metamorphosis, and is fundamental to the survival of the tadpole during this energetically demanding process. Tadpole liver and tail fin are heavily involved in the innate immune response and are valuable organs to study during metamorphosis as well as during disease challenge. Disruptions to vital TH pathways have negative implications for the intricate process of tadpole development, which presents the opportunity to use frogs as sentinels for research on TH disruption. A wide variety of endocrine disrupting chemicals (EDCs) have been shown to interfere with hormone signaling in vertebrates, including amphibians. These include chemicals from sources such as pharmaceuticals, herbicides, pesticides, and personal care products. Conventional wastewater treatment methods do not fully remove EDCs, allowing low concentrations to persist in treated wastewater effluent. These low level EDCs may remain biologically active and disruptive to the endocrine system of developing tadpoles. Disruptions to TH pathways that affect the development of innate immunity in tadpoles may result in increased susceptibility to disease and infection. In the present study, we exposed American Bullfrog (Rana (Lithobates) catesbeiana) pre-metamorphic tadpoles to the THs 3,5,3'-triiodothyronine (T3) and thyroxine (T4), the sex-hormone 17 beta-estradiol (E2), as a non-TH control, a cocktail of known EDCs, and treated municipal wastewater effluent. Gene expression was evaluated in the tail fin and liver using RNA-seq and targeted qPCR assays to determine the impact of these chemicals on various genes and pathways, including those relating to the innate immune system. It has been empirically demonstrated that the innate immune system of bullfrog tadpoles is affected by exposure to TH. The presented work will further our understanding of the connection between EDCs and innate immune system perturbation.

MP052 Gene Expression Responses in Freshwater and Marine Fish Exposed to Wastewater Effluents

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Laboratory exposures were conducted to determine the transcriptome response between freshwater and marine fish species after exposure to wastewater effluent. We performed microarray and gene network analyses in sexually mature male fathead minnows (Pimephales promelas) and marine, field caught, male hornyhead turbot (Pleuronichthys verticalis). Agilent 8 x 15K, one color arrays were used to determine gene expression. Fish were exposed to negative controls, estradiol (E2), and 5% concentrations of municipal wastewater effluents. Secondary and advanced primary treated effluents were used. Fathead minnow (FHM) liver gene expression showed differences among fish exposed to estradiol and effluent. There was no pronounced differentiation among fish exposed to the different effluent types; although, several fish exposed to advanced primary treated effluent clearly grouped together. Gene network analysis revealed that some genes differentially expressed in FHM exposed to advanced primary treated effluent were involved in specific processes. For example, FHM had cholesterol and drug metabolism pathways that were down-regulated after exposure to advance primary treated effluent. Furthermore, there was a significant relationship between subnetworks regulated by E2 and fish exposed to primary treated effluent. Marine hornyhead turbot fish exposed to effluent were mapped to the FHM pathways to compare responses in both species. After mapping the turbot genes to the FHM pathways the results suggested that the marine fish may have responded on a similar manner to the freshwater species after effluent exposure. For example, estrogenic, cholesterol and drug metabolism pathways were also affected by exposure to both effluents, but a different magnitude of effects was found in the livers of turbot exposed to advance primary treated effluent. The present study demonstrates that some of the responses observed after exposure to environmental contaminant mixtures (e.g., effluents) were conserved in both marine and freshwater species.

MP053 Hepatic proteome to account for the molecular mechanism underlying dioxin susceptibility in C3H/lpr and MRL/lpr mouse strains

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Dioxins cause various toxic effects through aryl hydrocarbon receptor (AhR) in animals with inter-species and strain differences in susceptibility. C3H/lpr and MRL/lpr are inbred mouse strains that are a dioxin-sensitive and -resistant type, respectively. However, the molecular mechanism underlying the different susceptibility still remains unclear. Here, we adopted a proteomic approach using two-dimensional electrophoresis coupled with matrix-assisted laser desorption/ionization time-of-flight/ time-of-flight (MALDI-TOF/TOF) mass spectrometry to clarify the difference in effects of 2,3,7,8-tetrabromodibenzo-p-dioxin (TBDD) exposure on the hepatic proteome between C3H/lpr and MRL/lpr mice. To confirm the induction of cytochrome P450 isozymes (CYPs) by TBDD treatment in each strain, we initially measured CYP1A1 and 1A2 protein levels and their catalytic activities. Results showed that at the dose of 10 µg/kg body weight, TBDD treatment increased CYP1A1 and 1A2 levels in both strains, but a more prominent induction was observed in C3H/lpr than MRL/lpr. This supports that C3H/lpr was more sensitive to dioxins than MRL/lpr. We successfully identified 40 up- and 17 down-regulated proteins in C3H/lpr and 7 up- and 10 down-regulated proteins in MRL/lpr by TBDD treatment. Interestingly, the proteins induced in C3H/lpr were involved in the metabolism of tryptophan and its metabolites as endogenous AhR ligands, suggesting that AhR is more activated by accelerated production of endogenous AhR ligands in TBDD-treated C3H/lpr than MRL/lpr. We also identified that proteins responsible for reducing the oxidative stress such as superoxide dismutase and peroxiredoxins were up-regulated in C3H/lpr mice by TBDD treatment. The present study reveals that the high dioxin-susceptibility of C3H/lpr strain may be associated with more activation of AhR signaling by endogenous AhR ligands and more efficient elimination of oxidative stress.

MP054 Next generation sequencing analysis to assess the effects of prenatal bisphenol A exposure on developing chicken embryos

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Bisphenol A (BPA) is an estrogenic chemical that has been used in various products. Large amount of its usage has resulted in the contamination of BPA in wild animals including birds. There is growing concern about the toxic effects of BPA in developing organisms, because the early life stage is highly sensitive to endocrine disrupters. However, there is less information regarding the effects of BPA on avian embryos. In particular, the effect on gene expression profile has not yet been clarified. We thus performed an in ovo injection test using chicken eggs and investigated the effect of BPA on the developing embryos by analyzing their hepatic transcriptome by next generation sequencing (NGS). Seventy five chicken (Gallus gallus domesticus) eggs were divided into five groups (n=15 per group); vehicle (corn oil) control, low (10 ng/g egg), moderate (1,000 ng/g egg), and high (100,000 ng/g egg) doses of BPA- and 17 β-estradiol (E_2)-treated (10 ng/g egg) groups. On the 2nd day post egg-laying, test chemicals were injected into the air sac. On the 21st day post egg-laying, the embryos were dissected and each of organs was collected and weighed. RNA solutions extracted from liver samples were used for NGS analysis. To identify differentially expressed genes, transcriptome data obtained from the NGS was analyzed by cuffdiff. Pathway and network analysis of the genes differentially expressed by BPA or E_2 treatment were carried out by KEGG pathway and STRING, respectively. Measurement of the liver weight showed an increasing trend in BPA- and E2-treated groups compared with the control group. Data analyses of NGS indicated that mRNA levels of 204, 4078, 1766, and 3006 genes were significantly changed by the treatment with the low, moderate, and high doses of BPA, and E_2, respectively. This indicates that more effects on the transcriptome were induced in the groups treated with moderate and high doses of BPA and E_2 than in the group treated with low dose of BPA. Pathway analysis revealed that BPA affected the DNA replication, cell cycle, and nucleotide metabolism in the embryo livers. Network analysis indicated that cyclin-dependent kinase 1, serine/threonine-protein kinase PLK1, peroxisome proliferator-activated receptor alpha, and angiotensinogen were identified as hub genes in moderate and high doses of BPA- and E_2-treated groups, implying that these genes may play central roles in the effects of BPA on developing chicken embryos.

MP055 Sex-dependent effects of prenatal bisphenol A exposure on liver transcriptome of rat offspring

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Bisphenol A (BPA) is one of ubiquitous endocrine disruptors in the environment. Many studies have shown that BPA causes multiple effects such as alteration of mammary gland development, sexual maturation, immune system function, behaviors, and detrimental effects on glucose homeostasis and insulin sensitivity. However, knowledge is still limited on the mechanism of transgenerational actions of BPA. In this study, we treated pregnant Wistar rats with low (50 ng/kg bw/day) and high (5000 ng/kg bw/day) doses of BPA on embryonic day 4-18 and investigated the effects on the liver transcriptome of postnatal day 0 pups. Hepatic mRNA levels of pups were analyzed by a paired-end sequencing with Illumina HiSeq 2500. The genes which had significantly different FPKM (fragments per kilobase of transcripts per million mapped reads) in BPA-treated groups from those in the control group (FDR < 0.05) were used for further analysis. Network and pathway enrichment analyses were performed by using Cytoscape and its plug-in. Results showed that prenatal exposure to BPA altered mRNA expression levels of genes involved in the metabolic pathways, peroxisome proliferator-activated receptor (PPAR) signaling pathway, cell cycle, and DNA replication in both male and female pups. BPA exposure suppressed the genes related to the fatty acid metabolism in females at low and high doses, but only high dose of BPA caused the effect in males. This sex-dependent transcriptome effect was supported by phenotypic alteration, showing a similar pattern in the increase of offspring's body weight. Interestingly, BPA exposure may decrease glucose uptake and increase insulin resistance in females, but increase glucose uptake and insulin sensitivity in males through the sex-dependent effects on mRNA expression of glucose transporter type 4, insulin receptor and its downstream gene, phosphatidylinositol-4,5-bisphosphate 3-kinase. Moreover, the up-regulation of sterol regulatory element-binding protein 1cmRNA and the down-regulation of PPARα mRNA and its target gene, carnitine palmitoyltransferase 1 in females may lead to the induction of lipogenesis and inhibition of beta oxidation by BPA exposure. These results suggest different modes of action of prenatal exposure to BPA between male and female offspring. Further analysis including biochemical assays and quantitative PCR as well as the expression at the protein level of these genes will be performed to confirm the results.

MP056 Sex-specific molecular responses in the hypothalamus may underlie differences in behavior following exposure to Phenanthrene in the fathead minnow

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Phenanthrene (PHE), a three-ring polycyclic aromatic hydrocarbon, is a common environmental contaminant. Most often formed through incomplete combustion, PHE can be created both naturally and anthropogenically. PHE concentrations can be elevated in aquatic environments through oil spills, atmospheric deposition and industrial wastewaters. PHE has been described to have a narcotic mode of action and the objective of this study was to determine if sub-chronic exposure would affect behavior. The second objective was to potentially determine the molecular basis for this response in the hypothalamus. Fathead minnow (Pimephales promelas; FHM) were exposed to 201.8 µg/L average measured waterborne concentration of PHE in a static-renewal bioassay over 7 wks. After exposure, individual behavior in a novel aquarium test was measured. Following this, fish were sacrificed, body measurements taken, and hypothalamic tissues excised for transcriptomic analyses. Condition factor of both sexes were decreased after PHE exposure, and gonadosomatic index was decreased in exposed females but not males. Behavioral tests indicated that male fish exposed to PHE were more active than reference fish; however, there was no difference between female control and treated fish. Microarray analyses identified that there were 1126 and 1105 probes differentially expressed in males and females, respectively. Sub-network enrichment analyses identified 160 and 94 pathways, in male and female hypothalamus, respectively. Thirty-one pathways were common to both sexes. These included swallowing and cardiovascular reflex. Of the 129 unique pathways altered in male hypothalamus, forward locomotion, pineal gland function, and grooming behavior were down-regulated 18 – 39%. Pathways unique to the female hypothalamus included estrogen receptor signalling pathway and dopaminergic system. This study supports a narcotic mode of action for PHE and highlights some of the pathways in the central nervous system that may explain, in part, differences in the behavioral response to PHE.

MP057 Using the molecular basis of olfaction in metamorphosing frog tadpoles as an indicator of thyroid hormone disruption in aquatic systems

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The American bullfrog, Rana (Lithobates) catesbeiana, is a model organism for studying thyroid hormone action in vertebrate species. Metamorphosis from an aquatic tadpole to a terrestrial frog involves reconstruction of nearly every tissue in the amphibian's body and is entirely dependent on the action of thyroid hormones, L-thyroxine (T4) and 3,5,3'-triiodothyronine (T3). Premetamorphic R. catesbeiana tadpoles lack any measurable circulating thyroid hormone and exogenous TH exposure results in a precocious induction of metamorphosis. Olfaction is a critical component of tadpole survival, facilitating food location and predator avoidance. Olfaction requirements change during metamorphosis as the herbivorous tadpole transitions from aquatic to terrestrial environments and becomes a carnivorous frog. Little is known about the influence of thyroid hormones or endocrine disrupting compounds (EDCs) found in household and industrial products on the olfactory system. In the present study, the olfactory impact of exposure to various hormones and known EDCs was investigated. Premetamorphic tadpoles were separately exposed to environmentally-and physiologically-relevant concentrations of T3, T4, 17 beta-estradiol (E2), and multiple concentrations of a chemical cocktail of known EDCs typically found in municipal wastewater. Further, municipal wastewater was spiked with this chemical cocktail and subjected to either anaerobic membrane bioreactor (AnMBR) or membrane enhanced biological phosphorus removal (MEBPR) processes. A parallel treatment train was operated for each with vehicle spiked into the wastewater influent. The olfactory bulb (OB) from the brain and the olfactory epithelium (OE) from the rostrum were dissected from tadpoles after 48 hours of exposure. RNA was extracted from the tissues and transcript levels were evaluated using RNA-seq and targeted qPCR. Gene expression data indicate that both tissues are responsive to thyroid hormones. The results will be compared with parallel studies performed on behavioral and neurophysiological endpoints to uncover important aspects of thyroid hormone-dependent processes in the olfactory system and the effects of disruption by anthropogenic EDCs.

MP058 Shotgun Proteomics of Hexagenia spp. exposed to Surface waters and Effluents from Toronto and Hamilton Harbour, ON

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Hexagenia are an ecologically and widespread group of mayflies belonging to the order Ephemeroptera. There is growing interest in the development of a standardized test protocol using Hexagenia for use in ecotoxicological assessments. Both Hamilton and Toronto Harbours were designated by the International Joint Commission as being Great Lakes Areas of Concern due to contaminated sediments from Industrial activity and excess nutrients from wastewater effluents. Our goal was to characterize the effects of exposure to surface water and effluents from these two areas on the Hexagenia spp. proteome. Surface water samples were collected from three locations in Hamilton Harbour and two locations in Toronto Harbour. Effluents were also collected from wastewater treatment plants (WWTPs) discharging into the Harbours. Mayflies were exposed to all effluent and surface waters for 48hrs, with 10 replicates per exposure. Each animal was analyzed individually. Shotgun proteomics is a method where the proteome is first digested into peptide mixtures and then characterized by LC-MS/MS. The largest number of protein matches were found in the Diptera (Flies) database, while the greatest relative number of species matches were found in the Odonata (Dragonflies& Damselflies) & Ephemeroptera (Mayflies) databases. Significant differential expression of common proteins among all exposure and control groups suggest that there were effects on the proteome of Hexegenia spp. by effluents. The discussion will identify potential ways that data from this study could inform regulatory frameworks.

Chemical, Biological and Instrumental Methods for Detecting Harmful Algae and Their Natural Toxins

MP059 Overview of USEPA Office of Research and Development's planned research on Analysis and monitoring in fresh and coastal/ estuarine environments

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Several factors are contributing to the development of the "perfect" Harmful algal Bloom (HAB) storm. For example, climate change associated with elevated temperatures over prolonged time periods, changes in population demographics, agricultural land use linked to nitrogen loading increases, chronic economic stress and an aging water treatment infrastructure all combine to increase the probability of toxins breaking through to consumers' taps. In August of 2014 the State of Ohio issued a "Do Not Drink" notice after a harmful bloom event when elevated levels of microcystin (≥1 μg/L) were detected in the finished water of a treatment system serving approximately 1,900 customers. This system drew raw water from a location in Lake Erie close to the intakes of the City of Toledo, which provides water to about 500,000 residents. Increases in salinity are also having adverse ecosystem impacts stemming from freshwater HABs and invasive toxic algae. In addition, high biomass blooms are known to have adverse ecosystem impacts such as reduction of the photic zone impacting sensitive trophic interaction and reduction of oxygen levels that kill fish and bottom dwelling organisms. Some non-cyanobacterial freshwater HABs also produce toxins that can kill fish. The most problematic of these is the marine invasive Prymnesium parvum (i.e., "golden algae") which has caused fish kills in Texas annually since 2001 and has been documented in at least 10 other states. As a result of the human, ecological, and economic impacts of HABs, the USEPA's Office of Research and Development has established a research program to address issues related to the detection, quantification and monitoring of algal blooms and the organisms that produced them. This research plan has several objectives: develop new or refine existing chemical, instrument and biological methods for the detection of cyanobacteria and their toxins; test such methods in field studies in both HAB and non HAB environments; determine the method(s) that can be best uses as early warning (pre bloom conditions within days to weeks) systems for the detection of cyanobacteria and their toxins. We will discuss ongoing efforts on research projects that include chemical detection of toxins, use of molecular, flow cytometry, mass spectrometric, and microscopic approaches for phyto/ zooplankton identification, Phone apps for HABs early warning, and advanced instrumental and hyperspectral image analysis approaches.

MP060 Analysis of total microcystins in water at low part-per-trillion levels by on-line solid phase extraction coupled to UHPLC-MS/ MS

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Anthropogenically-induced eutrophication of aquatic ecosystems, in conjunction with rising water temperatures worldwide, have been linked with increased frequency and magnitude of harmful algal bloom events. The proliferation of waterborne cyanobacteria may result in blue-green or red layers to form intermittently on freshwater or marine habitats, a harbinger of further risks to come when the bacteria release endotoxins upon dying. Among the wide panel of naturally-occurring cyanotoxins, microcystins are the most frequently monitored. In recent years, exceedances of the World Health Organization (WHO) drinking water guideline for microcystins (1 μg L^{-1}) have led to temporary "do not drink" notices, including in Northern America. A standard method for microcystin surveillance in drinking water facilities is the enzyme-linked immunosorbent assay (ELISA) that may achieve detection limits as low as 40 ng L^{-1}. Despite many acknowledgeable benefits, the ELISA approach has been reported to produce false positives, a potentially contentious issue for water managers who need to correctly and readily identify risks before taking the appropriate measures. In this context, a fast and reliable method is proposed for the quantitative determination of total microcystins in drinking water. After a Lemieux-von Rudloff oxidation step to yield the 2-methyl-3-methoxy-4-phenylbutyric acid (MMPB) moiety, samples were directly analyzed by solid phase extraction coupled online to liquid chromatography electrospray ionization triple quadrupole mass spectrometry. Particular care was devoted to method optimization, addressed through experimental designs to incorporate potential interactions between factors. Hence, the choice of on-line solid phase extraction settings was conducted using a multi-criteria desirability approach (Derringer's functions). This allowed for the ultrasensitive determination of total microcystins in water at sub part-per-trillion levels (method limit of quantification ~ 0.4 ng L^{-1} total microcystins, 2500 times lower than the current WHO threshold). Excellent determination coefficients were observed on the linearity range tested which covered > 4 orders of magnitude. Accuracy, recovery and precision were also evaluated and proved idoneous, independently of the spike level. Total microcystins are now amenable to ultra-trace analysis in drinking water resources.

MP061 Use of Membrane Filtration as an Alternative Method in the Extraction of Microcystin Cyanotoxins Produced by Harmful Algal Blooms

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The frequency and intensity of harmful algal blooms (HABs) is increasing for a variety of reasons, with implications for human/animal health and water use. The development of analytical tools to measure and monitor individual microcystin cyanotoxins produced by HABs is complicated by several factors, including a limited availability of commercial standards and uncertainty about the variability and toxicity of the diverse microcystin congeners. Current methods invariably require sample concentration, typically solid-phase extraction, so as to be amendable for measurement at ambient concentration levels. Such methods (i.e. EPA Method 544) are only validated for a limited number of the known variants where standards are available. However, the efficiency and validity in the use of solid-phase extraction cannot be adequately confirmed for other variants. This research investigates the use of membrane filtration as an alternative methodology for extraction and concentration of various microcystins.

MP062 Acute and chronic toxicity testing of algal toxins using a suite of aquatic test organisms

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Cyanobacteria, or blue-green algae, produce toxins that act as defense against grazers and competitors and that can also adversely affect aquatic and terrestrial animals as well as humans. Toxicity associated with fresh water cyanobacterial blooms was first described in 1878 and is now an issue worldwide. Cyanobacterial blooms have been increasing in frequency and intensity and have been linked to increased eutrophication from urbanization, agriculture and industrial nutrient enrichment. Microcystin and cylindrospermopsin are two of the most common classes of algal toxins found in waters of the United States. The objective of this study is to evaluate the toxicity of these two algal toxins to a diverse set of commonly tested aquatic invertebrates, fish, and amphibians. Species tested include the amphipod Hyalella azteca; the midge Chironomus dilutus; the mussel Lampsilis siliquoidea; the cladocerans Ceriodaphnia dubia and Daphnia magna; the fathead minnow Pimephales promelas; the rainbow trout Oncorhynchus mykiss; and the gray tree frog Hyla versicolor. Acute toxicity screens were conducted at nominal concentrations of 1 mg/L for microcystin-LR and 0.025 mg/L for cylindrospermopsin which exceeded the 90th percentile of ambient concentrations in U.S. surface waters. In addition, four species (H. azteca, C. dilutus, D. magna and P. promelas) were retested at four-fold higher concentrations (4.0 and 0.1 mg/L, respectively). Concentrations of algal toxins measured with Enzyme-linked immunosorbent assays (ELISA) averaged 98% of the nominal microcystin-LR concentration and 152% of the nominal cylindrospermopsin concentration. Survival in the first acute toxicity screen was ≥80% for both algal toxins and survival was 100% for the species retested at higher concentrations. Chronic 35-d exposures are being conducted with fathead minnows exposed to nominal concentrations up to 0.01 mg/L microcystin-LR and 0.0025 mg/L cylindrospermopsin. Results of acute toxicity screens indicates that concentrations of microcystin-LR and cylindrospermopsin reported for lakes and streams in the U.S. are unlikely to pose substantial risks of acute toxicity to aquatic organisms.

MP063 Cardiotoxicity of the blue-green algae Microcystis aeruginosa on developing Japanese medaka (Oryzias latipes)

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Microcystis aeruginosa (M. aeruginosa) is a freshwater cyanobacterium that is associated with harmful algal blooms worldwide. M. aeruginosa blooms are of particular concern due to the higher temperature optimum and nutrient source flexibility of cells, along with their increasing distribution and secretion of heptotoxins, microcystins, by toxic strains. Blooms are toxic to a wide range of organisms, including humans, but are potentially more detrimental to aquatic individuals due to their chronic exposures and multiple routes of entry for microcystins. Effects of M. aeruginosa have mainly been examined in adult and juvenile fish, although early life stages (ELSs) are possibly more susceptible to the toxic effects. Survival and growth of ELS fish may represent a bottleneck for sustaining healthy populations, making effects at this level have elevated ecological significance. We have been examining the toxicity of natural blooms and pure cultures of M. aeruginosa in addition to the pure toxin, microcystin-LR, on embryos of Japanese medaka (Oryzias latipes). Decreased heart rate was found to be the most sensitive endpoint assessed, as compared to survival, time to hatch, body length, and gross developmental abnormalities. Continuing work examines whether the observed bradycardia is due to structural changes or temporary depression of heart rate, and if observed effects translate to changes in respiratory capacity and bioenergetics of developing fish, which could have long term ecological implications.

MP064 Microcystin-LR in Mouse Liver Tissue: Extraction and Analysis

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Microcystins (MCs) are common global cyanobacterial toxins found in marine and freshwater environments. The adverse health effects they produce are primarily due to protein phosphatase inhibition in the liver. An important factor in assessing potential MC mammalian toxicity is the relationship of environmental exposure to hepatic toxicity and tissue levels in the liver of affected animals. With these data, environmental exposures can be directly compared to actual levels in the target organ, and these data increase the ability to evaluate comparative inter-species toxicity as well as MC levels in other human samples that may be available for epidemiology studies. This presentation covers only a fraction of the entire study and focuses on the method development on the extraction and analysis of liver tissues from mice dosed with known amounts of microcystin-LR (MCLR) as the representative congener. Several extraction approaches are currently being explored for best recoveries of free and extractable MCLR and other MCs as well as extraction approaches for total, free and bound MCs. Extracts are analyzed using an ultra-high performance liquid chromatography (UPLC) – quadrupole time-of-flight mass spectrometry (QToFMS) and a triple quad MS (MSMS) systems. Preliminary data show very low recovery of free and extractable MCs based on the amounts spiked to non-dosed liver tissues prior to extraction. These results imply two things: 1) the extraction method needs further optimization, and/or 2) most of the MCs are bound to tissues. These results are inconclusive at this point since the total MCs extraction method development is still underway. More conclusive data should be available during the time of this presentation.

Environmental Application of Cell-Based and High-Content Screening Assays for Monitoring Program and Risk Assessment

MP065 Using reporter gene assays to evaluate endocrine disruption in the rivers of three agricultural areas in South Africa

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South Africa is the largest user of pesticides in sub-Saharan Africa. Here we report on the endocrine disrupting effects agricultural chemicals may have on the aquatic environment. Three regions with different crops were selected. The Letsitele site grows a variety of tropical and citrus fruits, Viljoenskroon area is dominated by maize and the Lomati region is known for its sugar cane. Water and sediment samples were collected bi-annually from the rivers from 2011 to 2013. Water samples were acidified to a pH of 2–3 and stored at 4°C. Filtered water samples were extracted with a C18 solid phase extraction cartridge and eluted with methanol. Dried and ground composite sediment samples underwent pressurised liquid extraction with a 3:1 mixture of dichloromethane:acetone. Clean-up steps included solid phase extraction, and size exclusion chromatography. Oestrogen activity was determined by the recombinant yeast screen (YES) and the T47D-KBluc cells. The androgen activity was investigated with the MDA-KB2 cell line. All the assays use the principle of a reporter gene being expressed when a ligand successfully binds to the respective hormonal receptor however the YES is a colorimetric assay, and both mammalian cell assays are luminescent. The mammalian cell assays were used for evidence of both activation and inhibition induced by environmental extracts. Stimulation of the hormonal receptor in the cells causes expression of the firefly luciferase and an extract's response is expressed in terms of the relative response by the positive controls: oestradiol equivalents (EEq) or testosterone equivalents (TEq). Inhibition was compared to the control cells slightly stimulated with positive control. Viability assays were run concurrently with all endocrine assays. The T47D-KBluc assay was more sensitive than the YES. Oestrogenic and androgenic activities were detected in water and sediment samples from all three agricultural areas. Oestrogenic activity in the water from across all sites and seasons ranged from < DL–6.78±2.28 ng EEq/L and in the sediment between < DL–254.95±69.2 ng EEq/kg with the highest for both matrices from the maize farming area. No androgenic activity was quantifiable for the water samples from the maize district however one of its sediment samples also had the highest activation of the androgen receptor. When comparing the levels of the commonly used pesticides in each area to the bio-assay results, no discernible pattern was found.

MP066 PROTECT (Puerto Rico Testsite for Exploring Contamination Threats) – Mixture Toxicity Assessment and Identification in Groundwater from Puerto Rico

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Complex mixtures of chemicals that may pose a risk to ecosystem and human health are often present in aquatic environments. Feasible tools are needed to monitor aquatic environments and help identify chemicals that may cause adverse effects. As part of the NIEHS research center—PROTECT program, this study aimed to provide a fast, initial screening and assessment of the potential ecological and health impacts of Puerto Rico groundwater samples to reveal environmental contaminants and their potential contribution to health impacts. For the first sampling event, 15 samples were collected from selected wells and springs on the north coast of Puerto Rico. Organic substances in these samples were enriched via liquid-liquid extraction (LLE) and polar solid phase extraction (SPE). The LLE extracts were subjected to chemical analysis of phthalates on GC-ECD and chlorinated volatile organic compounds on GC-MS. The SPE extracts were subjected to both untargeted chemical screening on GC-MS and quantitative toxicogenomics assays that measured molecular toxicity based on endpoints—Protein Effect Level Index (PELI)—derived from temporal altered protein expression levels for 148 biomarkers targeting all known cellular stress response pathways including general, chemical, oxidative, protein and DNA stress responses. The resulting proteomics toxicity profiles/fingerprints allowed for multivariate statistical analysis to reveal the similarities and distances among these samples. Different molecular toxicity levels (overall average and stress pathway-specific PELI values) and distinct 3-D toxicity profiles were revealed among the samples. For example, the highest PELI value in protein stress was observed with the MIT well water, where the the highest number of chemicals were detected, including pesticides, anesthesia and phthalates. The POL well water showed the highest general and oxidative stresses. Correlation analysis between chemical analysis results and different toxicity endpoints showed that the occurrence of pesticides such as terbacil, atrazine and aldrin was significantly correlated with protein stress ($R > 0.8$, $p < 0.01$). This study demonstrated that this new toxicogenomics in vitro assay scheme and methodology platform can be an effective tool for groundwater monitoring, which can help identify chemicals that may have adverse health impacts and provide insights into potential toxicity mechanisms associated with complex mixtures of chemicals in aquatic environments.

MP067 Passive Polyethylene Samplers and in vitro Bioassays to Investigate Biological Effects of Complex Mixtures of Gaseous Hydrophobic Organic Contaminants

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Extensive research has established that particle-bound hydrophobic organic contaminants (HOCs) in air pose a serious health risk to humans but the biological relevance of the gaseous fraction, which is freely available to diffuse into biological tissue, is poorly characterized. Humans are exposed to gaseous air pollutants every day via respiration, dermal uptake, and dietary uptake from crops into which HOCs partition. In this study, passive polyethylene samplers (PEs), which accumulate truly gaseous HOCs over time via diffusion, were deployed in air throughout the greater Cleveland area for about 2 months. 9 sites, representative of urban, residential, and rural locations, were monitored. Extracts were analyzed by gas chromatography with mass spectrometry (GC/MS) for a suite of target compounds including polycyclic aromatic hydrocarbons (PAHs) and organic flame retardants (OFRs). Total polybrominated diphenyl ethers (PBDEs) were greatest at a site in downtown Cleveland, while total PAHs were greatest at a suburban, residential site east of the city. Aliquots of each extract were used to investigate biological effects of the extracted mixture via in vitro bioassays. To measure relative potency in activating the aryl hydrocarbon receptor (AhR), recombinant murine hepatoma cell line H1G1.1c3 was exposed to 10-point serial dilution curves of each extract and fluorescence readings were taken at 24, 48, and 72 hours. Preliminary results demonstrate that environmental samples induced fluorescence signals significantly above what was seen in response to a non-deployed, clean PE at all time points. Results from chemical and biological analysis at each site will be compared to determine whether specific site types exhibited distinct potencies, and whether concentrations of specific compounds or compound groups correlated with potency.

This study offers proof-of-concept for the use of PEs coupled with in vitro bioassays as a promising tool for measuring integrated effects of environmentally relevant mixtures.

MP068 Assessment of storage conditions for wastewater influent and effluent intended for in vitro bioassay analysis

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Receptor mediated cellular bioassays are becoming increasingly popular to screen surface waters for chemicals of emerging concern (CECs), including pharmaceuticals, personal care products, steroid hormones, and endocrine disrupting chemicals. Since water samples cannot always be processed and tested immediately after sampling, it is important to identify what storage and preservation techniques will ensure the integrity of samples for bioassay testing. The aim of this study was to investigate how different storage conditions, mainly bottle type, temperature, time, presence of a preservative, and presence of an oxidant quenching agent impacted the response of a CEC mixture using a suite of in vitro bioassays. Briefly, 250 mL of influent or 500 mL of effluent was placed in either amber glass or HDPE bottles. Sodium azide (NaAz) or sodium omadine (NaOm) were added to the bottles at a concentration of 1.0 g/L or 64 mg/L, respectively, along with a no preservative control. Sodium sulfite as an oxidant quenching agent was tested only with effluent samples. A mixture of 48 CECs was added to each bottle to a final concentration of 2 ppb for influent and 1 ppb for effluent. Samples were stored at 20, 4 or -18°C for 0 h, 24 h, 48 h, 72 h, 7 d, or 21 d, with all treatments and conditions tested in triplicate. At each time point, an aliquot from each bottle was taken for targeted chemical analysis, with the remaining sample being extracted by solid phase extraction with Oasis HLB cartridges. GR-UAS-blaHEK 293T and ER alpha-UAS-bla GripTite cell based assays (Invitrogen) were used to identify the glucocorticoid and estrogenic potential of the extracts. Preliminary bioassay results for effluent samples indicated that bottle type did not impact the response of the mixture in either assay, and the response was stable in samples stored at 20?C for up to 72 h, and for 3 weeks when stored at 4?C or -18?C without a preservative or with NaAz. The use of NaOm did impact the response of the glucocorticoid receptor assay, with the response being lesser in samples stored at 20?C for 72 h and at 4?C for 3 weeks. Analytical quantification of the water samples will be used to investigate the stability of each compound over time and as a confirmation of overall bioassay response tied to analytical values. Ongoing research involves testing the effluent sample extracts along with conducting additional bioassays to develop a standardized procedure to be used across laboratories.

MP069 Monitoring Ecological Impacts of Environmental Surface Waters using Cell-based Metabolomics

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Optimized cell-based metabolomics has been used to study the impacts of contaminants in surface waters on human and fish metabolomes. This method has proven to be resource- and time-effective, as well as sustainable for long term and large scale studies. In the current study, cell-based metabolomics is used to investigate the impacts of contaminants in surface waters on biological pathways in human and ecologically relevant cell lines. Water samples were collected from stream sites nationwide, where significant impacts have been primarily measured from the most potentially contaminated sources (i.e. waste water treatment plants, concentrated animal feeding operations, mining operations, and plant-based agricultural operations that use intensive chemical applications). Zebrafish liver cells (ZFL) were used to study exposure impacts on in vitro metabolomes. In addition, a small number of water samples were studied using two human cell lines (liver cells, HepG2 and brain cells, LN229). The cellular metabolites were profiled by nuclear magnetic resonance (NMR) spectroscopy and gas chromatography mass spectrometry (GC-MS). Detailed methods and results will be reported.

MP070 Cell-based metabolomics approach for assessing the impact of wastewater treatment plant effluent on downstream water quality

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Wastewater treatment plants (WWTP) are a known source of various types of chemicals including pharmaceuticals and personal care products (PPCPs), naturally occurring hormones, and pesticides. There is great concern regarding their adverse effects on human and ecological health through low-level, chronic exposure. In some areas where water resources are limited, the WWTP may be located in the same watershed as drinking water treatment plants (DWTP) and thus have a potential negative effect on downstream water quality. In this study, we investigated the possible impacts of contaminants as they travel from WWTP into DWTP in a water usage cycle by using cell-based metabolomics. Zebrafish liver cells (ZFL) were exposed to water samples collected along a river, where a WWTP was located 9 miles upstream of a DWTP. The sampling sites include upstream of WWTP, WWTP effluent discharging point, downstream of WWTP, drinking water intake place and the treated drinking water. After 48 hrs, the intracellular metabolites of ZFL cells were extracted. The polar and non-polar fractions of metabolites were analyzed by ^{1}H NMR spectroscopy and GC-MS, respectively. Multivariate statistical analysis revealed distinct changes of polar and non-polar metabolite profiles in response to effluent exposure from WWTP. The impact of effluent on the polar fraction of ZFL metabolome gradually diminished downstream of the WWTP and become non-significant at the drinking water intake location. However, the impacts of effluent on non-polar metabolome were still substantial for cells exposed to downstream WWTP sample and drinking water intake sample. ZFL exposed to treated drinking water did not exhibit significant changes of polar and non-polar metabolome compared to upstream WWTP sample, implying that the contaminants from WWTP effluent were efficiently removed in drinking water treatment processes. This study demonstrated the utility of cell-based metabolomics in determining the impacts of WWTP effluent on downstream water quality.

MP071 Nanomaterial Toxicity through a High Throughput Screening Approach on Mytilus Edulis Hemocytes

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Nano-scale products are being produced faster than current toxicity screening can keep up with, making it difficult to develop a universal regulatory policy. The use of in vitro assays and high throughput screening (HTS) addresses this growing demand for a rapid means of developing a comprehensive regulatory framework for engineered nanomaterials (ENMs). Under the project NanoReg II, funded by the European Union, the objective of our research is to provide a means in which nanomaterials can be incorporated into REACH guidelines. In this context, the aim of this study is to develop an HTS platform based on toxicity screening through a cell culture on marine mussel (Mytilus edulis) hemocyte cells exposed to different ENMs at different stages of their life-cycle produced by industrial partners. In addition to this, sublethal concentrations were selected in order to assess biomarker responses to the presence of ENMs and to develop a response profile. Future work will focus on characterizing the mechanistic pathways of toxicity for these ENMs and providing a comprehensive risk assessment and life-cycle analysis of products tested. This work is funded by the European project NanoregII (Development and implementation of Grouping and Safe-by-Design approaches within regulatory frameworks)

MP072 Estrogen-driven apical endpoints in Menidia beryllina

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The inland silverside (Menidia beryllina) is an established fish model for estuarine toxicity and is useful to determine effects of estrogenic chemicals on apical endpoints. Focusing on estrogenic chemicals commonly found in wastewater effluents, we exposed Menidia to increasing concentrations of 17β-estradiol (E2), estrone (E1) and 4-nonylphenol (4-NP) to determine changes in gonadal differentiation and growth, two important apical endpoints used for risk assessment. In vivo endpoints included alterations in molecular gene expression of two genes controlled by estradiol, vitellogenin and choriogenin. Growth was measured after 14 days and gonadal differentiation and sex ratio were measured after 28-days of constant exposure to the chemicals and after two months of transfer to clean water. Critical for the success of the experiment was to start the exposures before tissue differentiation in females and nutrition based on dechorionated artemia. Concentrations required for sex ratio skewing towards females was 200 ng E2/L and 100 ng E1/L. NP did not cause sex ratio skewing. All three of the chemicals caused retardation of growth. Some of the apical effects were still present in fish after 2 months in clean water. Early exposures to endocrine disrupting chemicals may change the nature of the population of this estuarine species.

Soil Contaminants: Fate, Bioavailability, Environmental Toxicology and Risk Assessment

MP073 Use of an in vivo rat model to evaluate relationships between bioaccessibility and bioavailability of nickel in ultramafic soils

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Nickel (Ni) is one of the leading soil contaminants found at former mining and smelting sites across Canada. Unfortunately, current deterministic generic soil quality guidelines often overestimate actual risk of toxicity via contaminated soil ingestion. Agencies are now turning toward probabilistic risk assessments by analyzing two key factors of soil Ni toxicity: the bioaccessibility and bioavailability. This study tested three hypotheses to relate findings of Ni bioaccessibility using the Solubility/Bioavailability Research Consortium (SBRC) method and Ni bioavailability using an in vivo rat model in a grouping of different naturally Ni elevated soils ("Ultramafic Soils"). Findings of this study indicated that with increasing Ni-compound solubility, there was increased absorbance in the small intestine of animals and thus an increase in the bioavailability of the Ni from soil. Second, Ni bioavailability was able to be adequately determined from urine content; however bioavailability was not able to be linearly correlated with bioaccessibility measurements. Lastly, while bioaccessibility was found to generally overestimate bioavailability, this relationship was not found to be consistent amongst the soils tested. Overall, findings demonstrate that the relationship of bioaccessibility and bioavailability of Ni in these soils are not straightforward. It is concluded that there are additional factors that may convolute this relationship, including speciation and mineralogy of the soil. However, research into bioaccessibility and bioavailability relationships continue to move toward probabilistic risk assessment.

MP074 Bioaccessible Nickel in Various Particle Sizes of House Dust from Communities Close to Nickel Mining and Smelting

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Indoor dust particles that settle on surfaces adhere upon contact to the hands and are ingested through hand-to-mouth behaviours. Ingestion of elevated levels of nickel from dust may be a significant source of exposure. Nickel accumulation in mammalian organs can lead to chronic inflammation, increasing the risk for cancer, especially from soluble nickel compounds. Bioaccessible nickel, the amount of nickel that becomes available for intestinal absorption as a result of digestion in the stomach, is likely a more accurate exposure measure than total nickel concentration present in house dust. For bioaccessible nickel, the role of particle size depends on the site of origin and the distribution of organic and inorganic nickel phases with their varying rates of dissolution and adsorption to dust. We are examining the relationship between particle size and nickel bioaccessibility in Sudbury house dusts. Samples were separated using plastic sieves into ranges of < 10 µm, 10-41 µm, 41-70 µm, 70-105 µm, 105-250 µm particle size fractions. Bioaccessibility was determined using SBRC glycine digestion, which simulates stomach pH and churning via shaking the dust with extraction fluid in a water bath at body temperature. Nickel concentration was determined using GFAAS, and multiple linear regression was used to analyze the data. Dust particle size and total nickel concentration together explain 88% of the variance in bioaccessible nickel concentration (adjusted $R^2 = 0.88$, $p < .001$). Dust particle size was strongly and inversely correlated with bioaccessible nickel concentration ($r = -0.66$), and moderately correlated with total nickel concentration ($r = -0.43$). Bioaccessibility enables adjustment of exposure estimates for risk assessment. Thus, the results of this study will help inform risk assessors on the contribution of indoor dust to total nickel exposure through ingestion, for populations close to nickel mining and smelting activities.

MP075 Using XANES to identify the effect of ageing, speciation and mineral association of nickel on its bioaccessibility in soil

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Changes in the speciation and bioaccessibility of Ni in soil columns spiked with nickel-sulphate-hexahydrate were measured over a 168 day period as a function of soil organic content and synthetic precipitation (leach) rate. X-ray Absorption Near Edge Structure (XANES) spectroscopy identified a rapid conversion of added $NiSO_4 \cdot 6H_2O$ to less labile phases post spiking because of surface sorption kinetics and precipitate formation. Soils with an average pH >7 contained 47-70% $Ni(OH)_2$/NiO within fourteen days, while only 37% of Ni was present as $Ni(OH)_2$/NiO at day 1 in soil with a pH < 7. Conversion to $Ni(OH)_2$/NiO slowed substantially after day 1 in all soils, due to slower ageing processes such as diffusion, incorporation into crystal lattices, and Ostwald ripening of precipitates. Bioaccessibility was significantly increased with the presence of increased SOM due to the higher proportion of exchangeable complexes with carbonate and organics. Bioaccessibility decreased at nearly identical rates (0.11-0.13% day^{-1}) in all columns. Leach rate did not significantly influence speciation, but Ni bioaccessibility was increased in wetter soils. Increased stability of Ni complexations and the formation of Ni precipitates are assumed to account for much of the reduction of bioaccessible Ni through ageing related mechanisms.

MP076 Evaluating the ability of Alyssum murale to extract aged nickel from nickel-enriched organic soils

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Port Colborne, Ontario, has an area of nearly 30km^2 that has been contaminated by emissions from a nickel (Ni) refinery that was in operation between 1918 and 1984. Elevated Ni concentrations in the surrounding soil are causing phytotoxicity and are suspected of reducing crop yield in some agronomic species. Common remediation techniques (such as 'dig and dump') are not feasible or economically efficient because of the large area that has been contaminated. Phytoremediation would be a sustainable alternative if its efficacy could be demonstrated on spatial and temporal scales. Nickel hyperaccumulating species are able to accumulate at least 1000 mg kg^{-1} of Ni in their dry biomass without succumbing to toxicity. Alyssum murale, a hyperaccumulator of Ni that is native to Ni-rich serpentine soils from Mediterranean Europe, is a species of interest for phytoremediation techniques. Tissue concentrations of Ni in shoots of A. murale have been determined using field contaminated soils in pot and field studies; however the spatial and temporal capacity of this species as a perennial crop to measurably reduce the concentration of Ni in soils has not been demonstrated. The uptake of aged Ni by wild-type A. murale from Ni-contaminated organic soils will be compared to extractable soil Ni as it is influenced by varying chemical and physical properties of soil, and sequential cropping. From this study, we will establish a hypothetical timeline for reduction in soil Ni concentration, for consideration in a site remediation plan.

MP077 Metal Mixtures in Soil: Testing the Concentration Addition Approach as a Risk Assessment Tool

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The risk assessment of metal mixtures in soils is critical to Canada's mining industry and regulatory system. The assessment of mixtures relative to impacts of mining and associated activities is of high priority for Environment Canada and Canadian base metal mining sites. There is limited data available on the toxicity of mixtures to soil organisms, particularly in field application. The proposed research will evaluate the validity of the concentration addition (CA) model in predicting the toxicity of a ternary metal mixture (As, Co, Zn). Toxic units (TUs) are widely used in the comparison of single compounds when assessing mixture toxicity. This study will use current regulatory TUs to predict toxicity of metal mixtures to test the CA model. Metal mixture impacts on higher plant species, barley (Hordeum vulgare) and tomato (Lycopersicon escultenum), will be assessed for growth parameters (root and shoot length; root and shoot dry weight) and metal uptake using field contaminated, reference, and artificial soils. Soils from Deloro, ON, contaminated with anthropogenic sources of metals, will be collected and used in the toxicity testing. The outcomes of this research will serve as a predictive risk assessment tool for mixtures and their interaction in soils at base metal mining sites in Canada, as well other similar contaminated sites around the world.

MP078 Risk assessment of heavy metal-contaminated sediment based on heavy metal speciation and extractability

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Sediments in navigational routes and estuarine channels are often found to contain significant level of heavy metals. For the beneficial reuse of dredged sediments in terrestrial environment, ecological risk by the exposure of heavy metals to terrestrial receptors should be assessed. However, the commonly applied approach of ecological risk assessment based on total metal content in sediment analyzed by exhaustive extraction may overestimate the risk, leading to excessive and costly treatment of the dredged sediment prior to reuse. Therefore, development of an ecological risk assessment method considering heavy metal bioavailability is needed for cost-effective and reasonable management of dredged sediment. Because of the uncertainties and intensive labor requirements involved in biological tests, chemical extraction methods are often applied to predict the potentially bioavailable fraction of heavy metals in sediments from heavy metal speciation and extractability. In this study, different chemical extraction methods including sequential extraction (SE), toxicity characteristic leaching procedure (TCLP) and a single extaction test (H_2SO_4 1.5 M, and HCl 0.5 M) were applied to a heavy metal-contaminated sediment sample dredged from one of the major ports in Korea. Ecological risk and contamination level of the sediment sample was charcterized from the heavy metal speciation using risk assessment code (RAC) and individual contamination factor (ICF) suggested in the literature. The amount of heavy metals extracted by TCLP and single extraction was greater than the sum of weakly bonded metal forms (exchangeable and carbonate bound fractions) from SE. RAC indicated high risk for Zn and Cd and low to moderate risk for Ni, Cu and Pb. ICF indicated that Zn and Cu posed a serious contamination while Cd posed a moderate contamination. Because different results and conclusions can be obtained from the various extraction and assessment methods, these methods should be more comprehensively reviewed in comparison with biological tests for the incorporation of heavy metal bioavailability to ecological risk assessement. Acknowledgement: This study recReived substantial support from the Geo-Advanced Innovative Action (GAIA) Project of the Korea Environmental Industry & Technology Institute (KEITI).

MP079 Overcoming Challenges in Assessing Risks to Wildlife at a Former Lead/Zinc Mine in the Yukon Territory

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Ecological Risk Assessment (ERA) studies were conducted at a former lead-zinc mine located in the Yukon Territory of Northern Canada. These studies were conducted concurrently with site characterization and other investigations to support permanent closure of the mine. While the mine only operated for 16 months in the early 1990s, metal concentrations in soils in areas of environmental concern (AEC) were well above applicable standards. Metal concentrations in terrestrial plants, invertebrates and small mammals were also elevated in contaminated mine site areas, relative to reference locations. Wildlife were evaluated in the ERA using scenarios and phased analyses to overcome several challenges: (1) ERA delivery alongside site characterization and closure planning, (2) spatially disconnected AECs and contributions from natural mineralization (3) supporting further understanding of the potential consequences to wildlife species of elevated metals concentrations. Phases of the wildlife ERA included: Phase 1: Modeling hazard quotients for 17 bird and 16 mammal species for multiple scenarios including: Current, predicted post-closure and alternative management actions, Mine site or smaller spatial scales, depending on receptor home range. Phase 2: Effects characterization (i.e., potential types and sizes of effects) using model-estimated doses compared to multi-study literature-derived dose-response datasets. Phase 3: Wildlife analysis assessing species presence and densities in various areas of the mine. Receptor and contaminant combinations showing potential risks were carried forward into subsequent phases of the ERA for further refinement of risk estimates. The scenario testing provided area-specific conclusions for directed management actions. Phases 2 and 3 characterized potential consequences including types and magnitude of effects, and number of individual organisms (for listed species) or proportion of mine site population (for common species) potentially affected. This phased and scenario-based approach to ERA provided managers, regulators and First Nations with supplementary information to support site closure decision-making.

MP080 Arsenic uptake by beets (Beta vulgaris) cultivated in a roxarsone-contaminated medium

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Arsenic (As) is a global toxicant that negatively impacts human health. Roxarsone (ROX) is an organoarsenical administered to poultry to control internal parasites. ROX is excreted from poultry unchanged and the waste may be used for vegetable fertilizer. This experiment was conducted with beets (Beta vulgaris) by adding 0, 1, 10, and 100 mg/kg As (T_1, T_2, T_3, and T_4 respectively, with ROX, presented as As concentrations) to a growing medium prepared with topsoil in a greenhouse. The study aimed to determine effects of As-contaminated soils on biomass production, uptake of As by beets, and allocation of As to tissues. Results showed that biomass production of beets was negatively correlated with As concentrations in the growing medium ($r = -0.3286$, $p < 0.0001$). As uptake by beets was positively correlated with As concentrations in the growing medium (roots, $r_s = 0.7577$, $p < 0.0001$; shoots, $r_s = 0.8406$, $p < 0.0001$). As uptake by beets was observed with median values in the roots of 0.267 ± 0.004 mg/kg, 0.271 ± 0.001 mg/kg, 0.271 ± 0.289 mg/kg, and 3.76 ± 1.92 mg/kg for T_1, T_2, T_3, and T_4 respectively; the shoots took up 0.259 ± 0.006 mg/kg, 0.263 ± 0.313 mg/kg, 0.271 ± 0.373 mg/kg, and 3.94 ± 0.72 mg/kg for the respective treatments. Beets took up 4.3 ± 2.3% of available As and distributed it equally into tissues. The results suggest that As could be transferred to humans through the food chain via beet consumption.

MP081 Tools for Development of Site-specific Clean-up Values Protective of Soil Ecological Receptors at Metal and Metalloid-contaminated Soils

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Until recently, there was no uniform guidance for developing site-specific Soil Clean-up Values (SCVs) for metals and metalloids in contaminated soils. Ecological soil screening levels (Eco-SSLs), although appropriate for screening, have been misapplied for soil remediation at contaminated sites. Using soil invertebrate, plant, and microbial toxicity data, available in the European REACH (Registration, Evaluation, Authorisation and Restriction of Chemicals) datasets, we present the tools that can be utilized for developing an SCV, using metalloid Mo as an example. These tools include accounting for the chemical bioavailability as a function of specific soil parameters of the contaminated site, correcting for differences in bioavailability between laboratory and field conditions, the development of Species Sensitivity Distribution (SSD) for ecologically relevant soil biota and critical soil processes, and review of the options for the selection of site-specific risk ranges for SCVs. A transparent generic approach is presented for the application of these tools and selection of appropriate effect thresholds (ECx) and hazardous concentration (HC) values (e.g., HC5 or HC50 protection level) to help site managers and regulators to derive an SCV resulting in an adequate protection of plants, soil invertebrates, and critical soil processes in a specific site.

MP082 Development and standardization of an ecotoxicological test method for the environmental risk assessment of genetically modified plants

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Before a genetically modified plant (GMP) may be released into the environment and placed on the market in the European Union (EU), an environmental risk assessment (ERA) according to EU directive 2001/18/EC must be performed. Currently, testing of the effects of GMPs on non-target organisms is mainly based on ecotoxicological test methods developed for the assessment of chemicals. This does not fully comply with directive 2001/18/EC, which demands a case-specific ERA. According to Annex II of the directive a 'case' is defined as a combination of the parent organism, its genetic modification and the possible receiving environment related to the intended release and use of the GMP. As the standard test organisms used for the assessment of chemicals do usually not occur in the receiving environment of GMP, they cannot be considered adequate. According to an ecology-based selection approach test species should be selected from organism groups relevant to the receiving environment and to the various exposure pathways. In addition, different taxonomical and physiological groups should be covered. Nevertheless, testing of any species in the laboratory also needs to be practical for a standardized application. Hence, the aim of a German R&D project (2012-2016) was the development and standardization of a laboratory ecotoxicological test especially for the ERA of GMP. This aim was reached in three steps. First, the black fungus gnat Bradysia impatiens Johannsen (Sciaridae: Diptera) was identified as a suitable test species and its mass rearing in the laboratory was established. The second work step comprised the actual development and trial of the test method that meets the above mentioned characteristics of the assessment of GMP. In the third and final work step the method was described in a draft guideline according to the specifications of the OECD. In this contribution said draft guideline and key results obtained during the method development will be presented.

MP083 Morphological, biochemical and yield response of Capsicum annum grown at different SLASH (sewage sludge and fly ash mixture) amendment rates

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Unplanned and improper disposal of solid wastes in most of the developing countries results in hazardous effects on environment. Agricultural utilization of sewage and fly ash mixture (SLASH) can be explored as one of a sustainable approach to manage these wastes. Land application of Slash can improve the physicochemical properties of soils and may help in promoting plant growth due to presence of beneficial plant nutrients. Slash mixture has more balanced properties, in which fly ash can serve as a potential stabilizing agent for sewage sludge by neutralizing it, killing pathogens, reducing toxic heavy metals availability and consequent damage to plants. The present study was conducted to assess the feasibility of agricultural utilization of SLASH mixture in terms of morphological, biochemical and yield response of Capsicum annum (Chili), a commonly grown household vegetable. Four amendment ratios of sewage sludge (SS): fly ash-(FA) (viz. 4 (SS):1 (FA), 4:2, 4:3, and 4:4 denoted as A, B, C and D, respectively) were mixed with the soil at two different rates of 20% and 40%. Significant increase in foliar protein content was reported in different Slash amendment rates with maximum increase in D40%. Peroxidase activity increased significantly in all the SS-FA amendments as compared to control plants except for C40% and D40%. Proline content was also found to increase insignificantly in most of the SS-FA amendments in Chili plants with highest significant increase at B40%. Morphological parameters and total plant biomass also increased significantly due to SS-FA amendments as compared to unamended soil. Maximum increment in shoot length, leaf area and total biomass was observed in amendment D20% and in root length and number of leaves in A20%. Yield also increased significantly across all the amendments with maximum increase of 94.6% at D20% followed by D40% (91.5%) and A20% (88.6%). Owing to positive morphological, biochemical and yield response amendments A and D can be recommended for Chili (Capsicum annum) plants

MP084 Advances in the Development of Procedures to Establish the Toxicity of Non-Extractable Residues (NER)

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The bioavailability and ecotoxicological relevance of Non Extractable Residues (NER) has been a major subject of debate for at least the past four decades. The debate focuses on the question, are NER an efficient detoxification process or are they an "environmental time-bomb" ? The ECO25 project sponsored under the CEFIC (European Chemical Industry Council) Long-Range Research Initiative research programme aims to shed some light on this subject. The principal deliverable from this research is to develop a scientific tool which is capable of responding to the demands of regulatory risk assessment needs to define the impact of NER. Because NER are not measurable in real life, the project has focused on the measurable fractions of the chemical in the soil compartment. New, but standardised extraction procedures are used to permit the evaluation of the ecotoxicity of fractions representing different degrees of bioavailability of test chemicals. It will also provide a clear, mechanistically driven, definition of NER and methodology to confirm the potential ecotoxic effects of NER. A series of ecotoxicity assays to determine the potential adverse effects of NER have been used including an Earthworm avoidance test, adaptation of the soil microflora toxicity test, Microtox^{Ò} screen and an acute Daphnia toxicity test. Three ^{14}C-radiolabelled molecules of distinctly differing chemistry and physicochemical properties have been investigated in three well characterised soils of varying composition. The intrinsic ecotoxicity of the chemicals in soils has been studied using the battery of assays by considering the effects immediately after application and then, after 6 months incubation (aging of the soil residue). The effect of formation of NER on the soil ecotoxicity of each test substance will be presented, discussed and interpreted in relation to current regulatory risk assessment of NER.

MP085 Measures of Chemical Availability for Enhancing Risk Assessments of PAHs in Soil

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A major uncertainty of many site-specific human health and mammalian risk assessments is the digestive bioavailability of Polycyclic Aromatic Hydrocarbons (PAHs) consumed via the incidental ingestion of contaminated soils. Relative to the bioavailability of PAHs used in spiked foods used in laboratory toxicity tests, soil-associated PAHs at contaminated sites are generally much less available. For example, laboratory tests that have measured in vivo exposures have noted that PAHs in soil contaminated with solid materials (e.g., soot) or weathered petroleum are many times less bioavailable compared to PAHs that have been freshly spiked with solvent or unweathered petroleum. This reduced bioavailability can be quantitatively accounted for in risk assessments using a Relative Bioavailability (RBA) value. However, laboratory feeding trials with live animals are expensive ($50,000 to $100,000 range or more) and require many weeks of experimental work. Fortunately, less expensive and more rapid chemical availability measures can be used to provide RBA information. This presentation provides an evaluation of two such tools: 1) Physiologically Based Extraction Tests (PBET) that extract PAHs from soil using a simulated digestive matrix; and 2) determination of freely-dissolved PAHs in soil via a commercially-available polyethylene passive sampling device. PBET and passive sampling results were evaluated for soils contaminated with solids-associated PAHs and compared to dose and RBA estimates for benzo(a)pyrene values generated from a laboratory experiment with rodents fed small amounts of the soils. Freely-dissolved PAHs data from the passive sampling measurements indicated that PAHs were approximately 10 to 100 times less available than PAHs spiked with unweathered petroleum and solvent, respectively, confirming the low availability of the solids-associated PAHs in the soils tested. Both chemical measures were positively correlated with benzo(a)pyrene bioavailability measurements obtained from the rodent feeding trial, indicating that empirical models may be able to be developed to relate chemical PAH availability measures to RBA estimates such that PBET and passive sampling data may be used to predict RBA values for use in risk assessments. PBET and passive sampling chemical measurements appear to be cost- and time-effective tools for predicting mammalian digestive PAH availability and improving the accuracy of site-specific PAH risk assessments.

Bioavailability of Organic Chemicals for Retrospective Risk Assessment: Measurement, Applications and Communication

MP086 How to communicate bioavailability science in regulation of organic chemicals?

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The bioavailability of organic chemicals in soil and sediment is an important area of scientific research. However, this area remains only partially recognized by regulators and industries working in the environmental sector. Based on the positive experiences already made with metals, regulators have recently started to consider bioavailability within retrospective risk assessment (RA) frameworks (e.g. of contaminated sites). By doing so, realistic decision-making in terms of hazard definition and priority considerations finally resulting in optimised cost allocation can be achieved, rather than relying on the established approach of using total-extractable concentrations. However, implementation of bioavailability remains difficult because scientific developments on bioavailability are not always translated into ready-to-use approaches for regulators and, therefore, no integrated approach for implementation is available. For the same reason, bioavailability remains largely unexplored within prospective regulatory frameworks that address the approval and regulation of organic chemicals. To facilitate the inclusion of bioavailability within more realistic RA frameworks, agreement and common understanding between scientists and regulators is required. In this identified communication need, we should try to answer the following questions: 1) What general message should be communicated, and which specific topics or benefits from bioavailability science can attract the interest of the regulators? 2) What is the best approach to convey these messages to the regulatory community? And 3) How can we establish a dialogue to verify that the messages are being considered and can be adapted to the satisfaction of all stakeholders? This contribution has been prepared by the six proposing authors that represent a bigger group of authors from academia, industry and regulation, who started this process, discussed bioavailability concepts and methods, and arrived at an agreement. The developments achieved at the Special Science Symposium focused on bioavailability, held in 2014 by SETAC Europe (http://sesss10.setac.eu/), led to a featured paper (Environ. Sci. Technol. 49:10255-10264, 2015). We propose a simplified approach in which the assessments of soil/sediment and the target chemicals should be based on two measurable values: the total extractable concentration, and the bioavailable concentration as measured with robust and reproducible chemical or biological methods.

Assessing Risks of Pesticides to Federally Listed (Threatened and Endangered) Species at a National Level – Part 1

MP088 Estimating the magnitude of effect using predicted pesticide exposure concentrations and toxicity data for an endangered species: A case study

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The U.S. Environmental Protection Agency (USEPA), the U.S. Fish and Wildlife Service (USFWS), and the National Marine Fisheries Service (NMFS), with assistance from the U.S. Department of Agriculture (USDA), are working together to develop an approach for evaluating risks of pesticides to species listed as threatened and endangered under the Endangered Species Act. Recently, a draft Biological Evaluation was completed for the organophosphate insecticide chlorpyrifos, which concluded that this chemical is likely to adversely affect individuals of the majority of listed species. Currently, the USEPA and Services are developing methods for assessing risks of chlorpyrifos to species at the population level. This population-level assessment will utilize exposure (i.e., modeled exposure concentrations) and effects (i.e., dose-response relationships) data to determine the magnitude of effect. Exposure concentrations are predicted on and near treated fields, including spray drift and runoff, for both terrestrial and aquatic habitats. Sublethal impacts to reproduction and behavior will be considered and are important for understanding population-level consequences of a pesticide exposure. A case example will be presented to show the predicted effects in both terrestrial and aquatic habitats for an amphibian species following labeled chlorpyrifos uses.

MP089 Approaches for Mapping the Habitat of Listed Species without Federally Declared Critical Habitat

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Spatially explicit sub-county habitat for federally listed species based on defensible scientific approaches allows for greater confidence and more realism in assessing the potential risk that pesticides have to listed species. Currently, refined spatial habitat information for a large number of listed species remains unresolved. Of the nearly 1,680 listed species in the US, approximately 788 have federally declared Critical Habitat suitable for use in national scale regulatory assessments. In the draft Biological Evaluations for three organophosphate compounds published in the spring of 2016, USEPA incorporated species range maps provided by The Services to make effects determinations for nearly all listed species; however, these range maps are not yet publicly available. This poster presents a study to explore approaches for efficiently generating spatial habitat using current data sources and techniques. While the habitat generated cannot represent "Critical Habitat", it should represent the physical and biological features occupied by the species. The goal is spatial data of appropriate accuracy and resolution for use with potential pesticide use sites in national-scale pesticide risk assessments. The science applied must be transparent and instill confidence in stakeholders that the data are representative and protective of the listed species. Federal Registry, USFWS reports, and NatureServe species profiles are highlighted as sources of textual habitat descriptions to inform both suitable habitat delineation and subsequent refinements to represent the often localized distributions of many endangered species. Tiered habitat maps are presented that show a tapering view from range to the biophysical parameters selected to represent suitable habitat. Several terrestrial, plant, and/or aquatic species across taxonomic groups are showcased.

MP090 Refined endangered species risk assessment for static aquatic habitats: Part 2 – Effects assessment and risk characterization

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The current USEPA approach to static aquatic exposure modeling at Step 2 of their interim process for national endangered species assessments uses simplified assumptions about water body characteristics, the surrounding landscape, and agronomic practices. At best, this approach may be considered suitable for screening purposes. A refined approach is proposed (Padilla et al. 2016 – Part 1) where 1000 30-year Pesticide Root Zone Model (PRZM)/Variable Volume Water Model (VVWM) realizations are applied that account for application timing, weather, soil and slope conditions, and crop configurations around ponds using probability distributions, by listed species. These VVWM realizations are then integrated with refined effect metrics (e.g., dose-response curves from appropriate taxonomic surrogates; species-sensitivity distributions) to generate refined risk estimates that incorporate differences in sensitivity between individuals or species. The use of the modeling along with other lines of evidence (e.g., monitoring data, incident reports, in situ toxicity studies, biological surveys) provides risk managers with the information and context required to evaluate the potential for pesticide risk to individual listed aquatic species. To demonstrate an approach, a case study for several species of aquatic invertebrates was developed and will be presented.

MP091 Environmental risks of synthetic pyrethroids in seawater

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Emissions of synthetic pyrethroids from salmon farms have been a concern. Pyrethroids are used as a chemotherapy alternative to control parasitic diseases in fish. However, environmental impacts of pyrethroids on marine organisms are still not well known under environmental conditions. Pyrethroids such as cypermethrin (CP) and deltamethrin (DE) used by salmon farms can be rapidly diluted in seawater after to be released, but its action mechanisms can be effective even with low concentrations on invertebrates, mainly on larvae stages. The objective of this work was design a method of risk assessment for several salmon farms treated with pyrethroids. Pyrethroid concentration measured in field and its effects on invertebrates of early life were compared using a probabilistic risk assessment method. Our study area was located in Chiloe Island (north Chilean Patagonia) where salmon farm activities are intensives. Passive water samplers were deployed around salmon cages during a treatment period with pyrethroid to determinate the measured environment concentration (MEC). For effect assessment, toxicity data collected from literature and bioassay in laboratory were included in the analysis. Physiological responses (clearance and ingestion rates) on Mytilus chilensis larvae were carried out in laboratory. Results showed that, passive samplers detected higher CP concentrations (MEC_{max}= 87 ng L^{-1}) than DE (MEC_{max}=3.2 ng L^{-1}) within a radius of 4 m around salmon cages for all salmon farms studied. In conclusion, probabilistic assessment suggests a higher risk of CP than DE exposed at invertebrate larvae in the marine environment. Acknowledgements: CRHIAM/Conicyt/Fondap N° 15130015; Fondecyt N° 1140466 project; Musels Millennium Nucleus (Ministry of Economy, Chile) NC 120086; (FIPA) Nº 2014-42 project.

MP092 What to do when predictions of risk models are at odds with real world observations: The Kirtland's warbler example for chlorpyrifos and malathion

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The United States Environmental Protection Agency (EPA) recently released draft endangered species Biological Evaluations (BEs) for chlorpyrifos, diazinon and malathion. As part of the draft BEs, EPA conducted refined risk assessments for selected listed bird species including the Kirtland's warbler (Setophaga kirtlandii). The Kirtland's warbler is an endangered migratory species that nests exclusively in young jack pine stands in Michigan and Wisconsin, and winters in the Bahamas. EPA's refined risk assessment for the Kirtland's warbler relied on the probabilistic Terrestrial Investigation Model (TIM) and Markov Chain nest productivity model (MCnest). Despite being probabilistic models, the models are hyperconservative in many aspects. For example, TIM assumes that Kirtland's warblers spend a significant portion of their foraging effort in and immediately adjacent to treated pastures during the breeding season. Decades of intense observation, however, indicate that Kirkland's warblers only forage in young jack pine forests during the breeding season and in similar habitats during migration. Because of their hyperconservative assumptions, TIM and MCnest predicted catastrophic mortality and near total reproductive failure for chlorpyrifos and significant adverse effects for malathion. No bird species, listed or otherwise, could withstand such effects without going extinct. The Kirtland's warbler, however, has dramatically increased in abundance in recent decades despite widespread usage of chlorpyrifos and malathion. This contradiction indicates that a more scientifically defensible modeling effort is required. We developed probabilistic, species-specific exposure and risk models to assess risks of chlorpyrifos and malathion to Kirtland's warblers during the breeding season and during spring and fall migrations. The breeding area model included several key information sources that EPA ignored in its draft BEs, e.g., species-specific foraging behavior, proximity of breeding territories to treated areas, and pesticide-specific data on prey concentrations. Similarly, the migration model took advantage of over a century of observations of when, where, and for how long Kirtland's warblers forage in different habitats during the course of their migration. The models found that chlorpyrifos and malathion pose negligible risk to Kirtland's warblers. These predictions are in line with the real world observations of the recovering Kirtland's warbler.

MP093 Developing population models for pesticide risk assessments: a systematic approach (for herbaceous plants)

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Population models have been recognized as important tools in pesticide risk assessments for species listed under the Endangered Species Act. However, not many population models for this specific purpose have been developed to date. A systematic, transparent approach to developing population models for pesticide risk assessment would ease the development of new models and the assessment and adaptation of existing models for this kind of risk assessment. We introduce a decision guide for the development of population models of herbaceous plants to be applied in pesticide risk assessment. The guidance walks the model developer through necessary steps that consider the specific questions to be addressed by the model, the life history of the species, and the data availability for species and habitat characteristics as well as pesticide exposure and effects. The guidance can either be applied to a single species or can be used to address a group of similar species. The resulting minimal conceptual model can serve as a blueprint for the implementation of a new model or to assess the applicability of an existing model for use in the context of pesticide risk assessment. Population- or species-level risk assessment is an extensive task given the number of species currently listed. Population models provide a tool to link population-level dynamics, species and habitat characteristics as well as exposure and effect information in a single approach. Developing such models in a systematic, transparent way will increase their applicability and credibility, reduce development efforts, and result in models that are readily available for use in risk assessments with varying compounds and exposures. We present the systematic approach for developing population models for herbaceous plant species and intend that the approach be adapted to provide model development guidance for other organism groups as well.

MP094 Endangered Species Risk Assessment Approaches for a Data-Rich Insecticide-Carbaryl

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National-scale endangered species risk assessments for crop protection products continue to be challenging due to the large amount of data and uncertainties involved in these analyses. The variety of use patterns, volume of environmental fate and ecological effects data, overall diversity and number of federally listed species, and other factors can substantially add to the complex nature of these assessments. At the same time, this volume of information greatly enhances the assessor's ability to increase the overall reliability and relevance of the risk assessment to listed species by allowing for more specific surrogacy assignments and exposure reflecting actual use patterns in potential proximity to species habitat areas. The insecticide carbaryl is an example of a well-studied crop protection product with decades of use experience and whose rich database allows for a pragmatic and detailed endangered species risk assessment. Like most products, biological sensitivity ranges are based on factors such as mode of action, receptor sites, metabolism etc. This information is crucial to understand differences in sensitivities between and within species taxa so that toxicity data can be better related to potential effects to specific listed species. Equally important is the thorough understanding of species habitats and locations so that exposure estimates can be accurately characterized. The full range of exposures and effects must then be uniquely related to use patterns, geographies and listed species for risk to be appropriately characterized to populations. In this paper, we will demonstrate examples of how this data can be collected, analyzed and applied to a national scale risk assessment for carbaryl. The pragmatic use of these best available data will allow for a more accurate and species focused risk estimate so that resources can appropriately be directed to stressors and listed species.

Environmental Risk Assessment of Metals in Tropical Regions: Challenges, Potential Solutions and Information Needs

MP095 Cu toxicity to cardinal tetras in Amazonian waters: blackwater, whitewater, particles, unusual DOMs, speciation, and modeling

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The toxicity of Cu to the tropical fish cardinal tetra (Paracheirodon axelrodi) was investigated in two major tributaries of the Amazon watershed: the Rio Negro (filtered < 0.45 µm, pH 5.6, DOC = 8.4 mg/L, Na = 33 µM, Ca = 8 μM) and the Rio Solimões (both filtered and unfiltered < 0.45 μm, pH 6.7, DOC = 2.8 mg?L^{-1}, Na = 185 μM, Ca = 340 μM), as well as in a natural "reference water" (groundwater) which was almost DOC-free (pH 6.0, DOC = 0.34 mg?L^{-1}, Na = 53 μM, Ca = 5 μM). Acute 96-h mortality, Cu bioaccumulation and net flux rates of Na, Cl^-, and total ammonia were determined in tetrasexposed in each water. Cu speciation in each water was determined via thermodynamic modeling and potentiometry (ion selective electrode, ISE), and Cu toxicity was predicted based on the Biotic Ligand Model (BLM) framework. Our results indicate that high Na loss is the main mode of toxic action of Cu in P. axelrodi, in accordance with general theory. Cardinal tetra showed a particularly high ability to tolerate Cu and to maintain Na balance (i.e. a low sensitivity), similar to the ability of this and other endemic Rio Negro species to tolerate low pH and ion-poor conditions. Both particles (high in unfiltered Rio Solimões water) and DOM (high in Rio Negro water) protected against acute toxicity and physiological disturbance. Overall Cu toxicity was lower in Rio Negro than in the other waters (at the same dissolved[Cu]). Indeed the free [Cu^{2+}] at the LC50, as determined by direct ISE measurement or speciation modeling, was approximately 10-fold higher. This variation could not be captured by a realistic set of BLM parameters. At least in part, this observation may due to gill physiological alterations induced by the unusual dissolved DOM of the Rio Negro (FAPEAM, CNPq, ADAPTA, Science without Borders, NSERC Discovery, ICA/SETAC Chris Lee Award, UBC).

MP096 Copper toxicity to Mekong and Lancang River organisms: development of a tropical Cu-BLM and application for setting Cu water quality guidelines

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Mekong River is the longest river in Southeast Asia (4,909 km) and is important to 60 million people in the four Lower Mekong River Basin countries (Cambodia, Laos, Thailand, and Vietnam). The recent industrial development, such as hydropower plants, mining, and metal processing along the Mekong River including the Upper River Basin in China poses potential metal contamination to the aquatic ecosystem of the Mekong River. A collaborative project between Loyola University Chicago, three universities in Asia, and Windward Environmental LLC on copper (Cu) bioavailability and toxicity to Mekong River and Lancang River organisms were conducted using field-collected waters in the past four years. The goals were to understand the sensitivity of Mekong River and Lancang River organisms to Cu, the influence of water quality parameters of Mekong River water on Cu bioavailability and toxicity, and to calibrate the US Cu-BLM to tropical environments in support of development of Cu water quality guidelines for the local ecosystems. In general, Mekong River organisms are sensitive to Cu. Copper toxicity decreased when water quality parameters, such as hardness (15 – 122 mg/L as $CaCO_3$), pH (6.39 – 8.30), and dissolved organic carbon (0.5 – 21.3 mg/L) were increased. The median lethal effect concentration ranged from 2.8 μg/L Cu (Ceriodaphnia cornuta, Vietnam) to 2,365 μg/L Cu (Ctenopharyngodon idellus, China). Experiments were also conducted to determine median lethal accumulation and critical values for Cu and Mekong River organisms. The US-Cu BLM was applied to the data set that was generated from the present study. In general, the US-Cu BLM did not predict well the toxicity of Cu to Mekong River organisms. However, using critical values for Cu and Mekong River organisms, the BLM predicted toxicity was strongly correlated with the measured toxicity. A species sensitivity distribution was also developed and will be used for setting Cu water quality guidelines for the Mekong River ecosystem.

MP097 Copper toxicity to tropical organisms: Are guideline approaches from temperate climates consistent with and protective for tropical species?

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The process of developing guidelines for the protection of aquatic life is dependent on an understanding of the sensitivity of organisms to a given toxicant, as well as an understanding of the primary factors that affect toxicity. In North America and Europe a considerable amount of research has been directed towards understanding factors that affect the bioavailability and toxicity of metals, with the recognition that water quality characteristics such as pH, hardness, and the presence of natural organic matter (NOM) can modify metal toxicity and therefore should be considered in the development of regulatory guidelines for metals. These toxicity modifying factors have been formally considering using a computation approach called the Biotic Ligand Model (BLM). The BLM is currently used in the United States (U.S.) and the European Union (E.U.) as a method for considering water quality in the latest regulatory standards for copper in freshwater. A marine BLM for copper is in review in the U.S. for setting standards in marine and estuarine waters. While the BLM has been developed to be a general approach that can be used in any region, there are important questions that need to be addressed to consider whether it is applicable in tropical regions. For example, if NOM is an important toxicity modifying factor, are there differences in the nature of NOM from tropical regions that require a revised modeling approach? Are aquatic species in tropical regions similarly sensitive to metals compared with organisms in temperate regions? Can species sensitivity distributions that have been compiled to support the development of guidelines in temperate climates be used to inform risk assessment and regulatory approaches in tropical regions, or are new distributions based on local species required? In this presentation, we will begin to address these questions by compiling information on copper toxicity to freshwater and marine organisms for tropical waters. These toxicity data will be compared with species sensitivity distributions used in the U.S. and E.U. We will discuss whether water quality guideline approaches that have been used in temperate countries would be adequately protective for sensitive species in tropical regions.

MP098 Tropical environmental risk assessment research program for nickel: Data gathering in Southeast Asia and Melanesia

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Global nickel production occurs around the world, and is increasingly dependent on lateritic deposits that occur in tropical regions such as Indonesia, New Caledonia, the Philippines, and Papua New Guinea. Refined environmental risk assessment approaches for nickel have been developed for temperate regions like Europe, where a bioavailability-based Environmental Quality Standard has been adopted under the European Union's Water Framework Directive. Applying the underlying ecotoxicity data and bioavailability models to tropical ecosystems is uncertain because of important differences in geochemistry, key taxonomic groups, and organism physiology between temperate and tropical systems. A broad research initiative was launched to address data gaps within the components of environmental risk assessment, including problem formulation, exposure and effects assessment, and risk characterization. Southeast Asia and Melanesia (SEAM) were chosen as the focus of this project because they represent the region of greatest tropical nickel production. The outcome of this research program will deliver the tools necessary for the nickel industry and regional regulatory authorities to perform advanced, refined environmental risk assessments of nickel in the SEAM region. A conceptual model was developed that identified key habitats of concern that included biologically structured ecosystems such as coral reefs, mangroves, and seagrass beds. Questionnaires were sent to regional stakeholders, and after QA/QC analysis, these resulted in a representative dataset covering the region. In terms of effects assessment, the existing literature was queried; high quality marine chronic ecotoxicity data were limited to three species of microalgae, one species each for echinoderms and anemone. In response to the paucity of existing high quality chronic ecotoxicity data, testing has been performed on corals, gastropods, crustacea, and fish. The next steps in the assessment process will be to validate effects thresholds based on laboratory results with higher ecological tier information, and to initiate a tiered risk characterization process based on well-characterized exposure scenarios.

MP099 Metal Fate and Transport in a Tropical River during Wet (Monsoon) Period: A Tale of Ligands domination

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Persistent and non-biodegradable metal contamination critically impacts riverine systems and dependent ecosystems through bioaccumulation and potential toxicity. In the river systems of developing countries in particular, metal contamination is a major water quality issue aggravated by rapid urbanization with its consequent copious runoff.We report aspects of metal fate and transport, seldom assessed in densely populated Brahmaputra River system in Assam, India with concomitant leaching escalating groundwater system vulnerability. The distribution of copper (Cu), lead (Pb) and zinc (Zn) between the dissolved and particulate phases of the Brahmaputra River, as well as chemical speciation and covariance with water quality parameters, was determined during wet (monsoon) periods in July 2014. Physicochemical properties of the water were characterized for geochemical speciation modeling using MINTEQA2. Free anions, cations and carbonates were dominant in the inorganic fractions and Cu, Pb and Zn concentrations were negligible in the anionic inorganic fractions. Metals were substantially higher in the particulate fractions than in the dissolved phase, attributable in part to high sediment loading during periods of high rainfall. Partition coefficients show high adsorption of Cu (3.1~6.1), Pb (3.4~6.5) and Zn (3.5~6.9) on suspended matter. Q-mode hierarchical cluster analysis (matrix of distances) of sampling locations illustrated associations governed by water quality parameters rather than the river course. R-mode (similarity) analysis implies affinity of the metals for ligands present in the water and suspended matter. Health risk index values were less than 1 for dissolved Cu, Pb and Zn but greater than 1 for total Pb and Cu, indicating potential human health risk. The binding of metals to naturally occurring dissolved organic matter or suspended particulates likely affects their bioavailability in the river during wet periods when sediment load is high. The combined empirical, computational and statistical analyses increase the understanding of the occurrence and speciation of metals and their interactions within a river system. Overall, metal fate and transport seems to be a tale of ligand domination and thus should be regulated through the proper discharge control of ligands.

MP100 Risk assessment for human health of fish consumption in two estuaries of Brazil

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Fishing is an important source of income for the population from estuaries of southern Brazil and has experienced difficulties arising from the contamination of the region mainly related to human activities. The yellow catfish (Cathorops spixii) is an abundant and economically important fish resource consumed by local population. The current study aimed to estimate the potential risk of metals (Pb, Sn and Hg) and arsenic (As) for human health through the ingestion of fish sampled in two important estuaries from southern Brazil. Fish were sampled in two sites of two estuaries from southern Brazil, Paranaguá and Guaratuba, during the summer/2013. Metals and As accumulation in muscle tissue were quantified and compared against national and international action levels regarding human consumption. In addition, the target hazard quotient (THQ) for metals and As, the cancer risk (CRisk) for As, and the number of safe meals per month were estimated. Among elements evaluated, As was accumulated at concentrations above action levels for human consumption in fish from all sampling sites (ranging 1.55 to 8.54 mg/kg). Hg was also detected in fish muscle. Highest THQs were estimated for fish collected in Paranaguá. In addition, CRisk above 10E-5 were estimated in all sampling sites. In a daily consumption scenario (356 days per year), it was possible to note risk to human health and CRisk in all sampling sites from both estuaries. In a weekly consumption scenario (56 days per year), the health risk was observed at one site of Paranaguá, however CRisk were high at both sites of Paranaguá and at one site of Guaratuba. Regarding the number of safe meals for adults, values lower than 16 meals per month were observed for one site of Guaratuba and one of Paranaguá. However, for children, lower rates of safe meals were observed for one site of Guaratuba and both sites of Paranaguá. In this study, it was possible to observe the accumulation of metals and As in fish muscle and possible consequences of this exposure to population from Paranaguá and Guaratuba. This exposure mainly to arsenic is primordial information to be included in the management of estuarine areas from southern Brazil. As observed, a daily consumption (even weekly for Paranaguá) of fish sampled in both estuaries sets up a risk for human health.

MP101 Preliminary investigation into the risk of environmental lead on the I.Q of pre-school children in Isiagu, Ebonyi State, Nigeria

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Isiagu town of Ebonyi State Nigeria is known for lead (Pb) mining and Pb is one of the oldest and most studied toxicants known. Its neurotoxicity is the most serious and has deleterious effect in children. Research on the relationship between the presence of low levels of blood lead in children, as a result of that found in the immediate environment, and a decrease in their cognitive-behavioral function, has been a source of controversy within the research literature; some researchers have argued that even very low levels of lead can have severe consequences on children's intellectual and academic functioning. In this preliminary human health risk assessment study, we carried out a proactive assessment of this issue by adapting the principle of bioaccumulation factor, to evaluate the consequences of environmental lead (Pb^+) in pre-school children in Isiagu town of Ebonyi State, Nigeria. This is a step in environmental and human health case study (project) being planned for the State. The sturdy examined the levels of Pb^+ in Isiagu ambient (air) environment at different intervals of the year, including that in the vegetables and crops mostly consumed by the community. Pre-school children numbering120, born within this community, aged 3-5 years, from three different pre-primary schools, also within this community constituted the sample for the cognitive function tests using Raven's Standard Progressive Matrices (SPM) while the control were children from two neighbouring communities, where no mining activity of any type had occurred. The data generated were analysed using (SPSS) version 21.0 and results expressed as mean ± standard deviation. The results were converted to intelligent quotient (IQ) using psychometrics. Students' t-tests for independent samples for the lead mining area and non mining area were used to compare the means. Pearson Correlation test was used to assess association between lead concentrations in the environment, in food crops and IQ of the children. P-value ? 0.05 was considered as significant (two-tailed analysis). The result of this investigation showed that environmental lead in Ishiagu was significantly high, as well as in the crops sampled, and the average IQ of pre-school children in this community was significantly lower than that in the control. This suggests that environmental lead could affect cognitive function in children and calls for an in-depth study involving blood sampling and other parameters.

MP102 Assessment of heavy metals in tropical soils of Mayabeque and Havana provinces, Cuba. Challenges and information needs

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Cuba is mainly an agricultural country and food production and quality depends on the health of the soils. Although authors of several studies proposed quality reference values of heavy metals (HM) in different soils that face little human activity, HM are not yet regulated in Cuba. In a joint research project, funded by the Swiss National Science Foundation, between the animal and plant health institute CENSA from Cuba and Agroscope ISS from Switzerland agricultural soils were monitored to assess HM concentrations. The study was performed in the Mayabeque province, Cuba where the soils mostly belong to agriculture and the Havana province that represent urban soils. Mean HM values (mg Kg-1dry weight (dw)) of 39 sites in Mayabeque were: Cd (2.6), Cr (63), Cu (64), Ni (48), Pb (16), Zn (55) and Hg (0.09). A principal component analysis suggested that the concentrations of Cd, Cu, Zn, Pb and Hg originated from anthropogenic activities, Ni was mainly from lithogenic sources, and Cr derived from both, anthropogenic and lithogenic sources. Mean HM concentrations (mg Kg-1dw) of the 22 sites in Havana province were: Cd (0.8), Cr (71), Cu (134), Ni (107), Pb (138), Zn (170) and Hg (0.04). The concentrations were, for most HM, higher than the ones of Mayabeque province. The soils around the port of the capital that reflects highest industrial activities including traffic had the highest HM concentrations. Also, in agricultural soils of Havana province the concentrations of Cu (195), Pb (197), and Zn (118) were higher than those found in agricultural soils of Mayabeque: Cu (54), Pb (19) and Zn (59). According to Dutch regulations, the concentrations of Cu, Pb and some of Zn in agricultural soils in Havana province clearly exceeded the target values (in mg Kg-1dw Cu 36, Zn 140, Pb 85) that indicate potential risks to ecosystems. This study serves as a basis for the Cuban government to establish recommendation values and to develop HM regulation in the Mayabeque province. The study results were communicated to decisions makers and stakeholders. Furthermore, a soil monitoring in other provinces is planned to create a soil monitoring network. It will provide important information for a better understanding of the fate, behavior and risk of HM in Cuban soils.

Wildlife Ecotoxicology: From Food Chain Exposure to Population Effects

MP103 Fox Too Are Not Spatially Relevant at Contaminated Sites

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Ecological risk assessments (ERAs) should only evaluate those species that are spatially relevant to their contaminated environs. To that end, ERA practitioners should seek out opportunities to establish that species they may believe to be of interest, actually satisfy a key, but often overlooked receptor-of-concern selection criterion, namely a demonstrated high site affinity. The use of newly available, state-of-the-art GPS tracking equipment to investigate the worthiness of the inclusion of gray fox (Urocyon cinereoargenteus) in terrestrial assessments, brings forward exposure information of great value. As with the outcome of a similar spatial movements tracking effort for White-tailed deer (presented at a SETAC N.A. meeting a few years ago), gray fox will probably never be appropriate for consideration within an ERA setting. The acquired high-quality actual (as opposed to modeled) exposure data of this study contributes to a growing and persuasive thinking on the role of (i.e., need for) mammal inclusion in ERAs.

MP104 HexSim Networks: Spatial IBMs are no longer just for the birds

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Legally protected animal populations are exposed to pesticides, the type and concentration of which vary extensively across space and time. Individuals from these populations are also simultaneously subjected to multiple other human-caused disturbance regimes such as landscape change, impacts from invasive species, and climate change. Our research community has relied heavily on models to quantify the impacts that pesticides have on wildlife, but to-date such models have dramatically simplified spatial structure and temporal dynamics, have had an organismal (not population) focus, and have ignored stressor interactions. While such simplifications have historically been born out of necessity, recent developments in simulation modeling are relaxing these constraints and changing what constitutes the "best available science". HexSim (www.hexsim.net) was developed specifically to quantify the impacts of multiple interacting disturbance regimes on wildlife and plant populations and this model can incorporate as much or as little detail about the study system as desired, including species life-history information, the distribution of dynamic interacting stressors, and effects due to other species. HexSim has recently been extended to include a complete suite of tools for simulating populations in river networks, making it a suitable platform for evaluating the impacts of pesticides and other anthropogenic disturbances on aquatic populations. This presentation will provide an overview of HexSim, targeting a SETAC audience, and then introduce our new HexSim networks toolkit.

MP105 Derivation of an Aquatic-Dependent Avian Tissue Residue Value (TRV) for Mercury Based on Current Toxicity Literature

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Mercury contamination of aquatic ecosystems is widespread and bioaccumulation of methylmercury through the aquatic food web potentially places predatory organisms at risk. Aquatic-dependent avian species appear to be particularly sensitive to mercury exposure, experiencing population level effects such as reproductive impairments at environmentally relevant concentrations (Burgess and Meyer 2008; Brasso and Cristol 2008). For most evaluations the results of several mallard studies (Heinz 1974, 1976a, 1976b, and 1979) were determined to be the most appropriate toxicity endpoints for the derivation of an avian Tissue Residue Value (TRV). These mallard studies demonstrated that exposure to dietary methylmercury reduced reproductive success at 0.5 ppm methylmercury (Heinz 1979). Recent studies suggest that mallards are less sensitive to mercury exposure than previously thought and may not be the most appropriate surrogate for some aquatic-dependent avian species. Additionally, current toxicity literature present lower toxicity endpoints (i.e., reproductive impairments at concentrations below 0.5 ppm) for sensitive aquatic-dependent avian species. This presentation discusses the derivation of a new draft TRV for aquatic-dependent avian species using the latest scientific literature focused on the effects of mercury.

MP106 Hepatotoxic Biomarkers in Crocodylus moreletii Adults from an Urbanized Lagoon in Tabasco, Mexico

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Crocodiles living in urbanized environments are exposed to a number of toxic compounds, which can produce harmful effects. Cholinesterases (ChEs) as acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) are used as effective biomarkers of contamination. Transaminases as GOT (AST), GPT (ALT), GGT and ALP are of great importance to reinforce the diagnosis of tissue damage. Studies about enzimatic biomarkers in C. moreletii are scarce, which is why the aim of this study was to evaluate the exposure to hepatotoxic contaminants through differences in biochemical parameters of tissue damage and health status between a wild population living in an urbanized lagoon and another kept in captivity. We captured reproductive females and males of C. moreletii (22) from both populations: wild (Laguna) and captive (CICEA). Blood samples were obtained by puncturing the occipital sinus with syringes previously washed with EDTA-K_2, samples were maintained in heparinized Vacutainers at 4 °C. Transaminase activity was estimated by Reflotron analysis, cholinesterase activity was measured by Ellman's method (1961), measurement of albumin was made using green bromocresol and total protein was estimated by Biuret's method. Albumin levels of Laguna's crocodiles were always lower than those from CICEA, this has been related to poor nutritional status or hepatitis in other organisms. Females from Laguna presented the lowest protein count, which has been related to immune deficiencies and liver disease in humans, indicating that females of Laguna could potentially be suffering of these conditions. The average AChE and BuChE activity was higher in Laguna than in CICEA. In wild organisms, GOT and GPT activity are much higher than in organisms from CICEA, AP activity was higher in Laguna than in CICEA and GGT levels are similar in males and females from both, wild and captive, groups. GOT and GPT are highly linked to liver damage, and GOT is strongly related to necrotic liver damage, indicating increased tissue damage. ANOVA analysis showed that AChE, BuChE, GPT and GOT levels are significantly different between CICEA and Laguna, while GGT and FA are not, moreover, these differences were not related to the sex of the organisms, and therefore, Laguna organisms may be exposed to contaminants that could be causing chronic damage in breeding crocodiles.

MP107 Acetaminophen toxicity in reptilians; underlying biochemical pathways for use of controlling invasive species

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It has been known for a while that some snake species are very sensitive to acetaminophen, the active compound in Tylenol® and other commonly used pain treatment medications. On the island of Guam, this knowledge is used to control the population of an invasive snake species: the brown tree snake (Boiga irregularis). It has also been proposed to also use acetaminophen to control the invasive Burmese python (Python bivittatus) in South Florida, but concerns about toxicity to other indigenous snakes and other reptilians has prevented any field applications so far. Little is known about the underlying mechanisms of why snakes are so sensitive to acetaminophen. We set out to investigate this, by measuring the expression and activity of enzymes that are involved in the biotransformation of acetaminophen in a variety of snake species, and some other reptilians, in comparison to several mammalian species. Reptilian liver samples were obtained from other researchers investigating these species, and were tested for enzymatic activity of sulfotransferase (SULT), UDP-glucuronosyltransferase (UGT), glutathione S-transferase (GST) and N-acetyltransferase (NAT), using acetaminophen-related compounds as substrates. The results showed that snakes had virtually no UGT activity, and very little NAT activity. This combination will lead to accumulation of aminophenol, a toxic metabolite of acetaminophen, which is known to cause methemoglobinemia in cats, and has also been observed in experiments with the brown tree snake. Enzyme expressions were consistent among the snake species tested. Screening of published snake genomes also suggested that these species do not have a functional gene for the UGT isoform that is responsible for acetaminophen biotransformation in other vertebrate species. Interestingly, American alligator (Alligator mississippiensis) and snapping turtle (Chelydra serpentine) did have UGT activity, although much lower than mammalian species like rat and cow. In conclusion, differential expression of Phase 2 biotransformation enzymes in reptilian species does explain their sensitivity to acetaminophen, and can be used to design management strategies for invasive species.

MP109 Re-evaluation of Osprey (Pandion haliaetus) Productivity and Contaminant Exposure in the Delaware Bay and River: Comparison between 2002 and 2015

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The last large-scale ecotoxicological study of ospreys nesting in Delaware Bay and River was conducted in 2002. Eggs collected in the northern region (C&D canal to north Philadelphia) had greater concentrations of organochlorine pesticides, PCBs, perfluorinated compounds, and PBDEs compared to the central Bay and southern Inland Bays. While reproductive success did not differ among regions, the probability of egg loss from nests was related to levels of halogenated pollutants, suggesting contaminants were a stressor. In 2015, ospreys nesting in the same regions were monitored to examine in productivity and pollutant exposure. A total of 27 nests were visited at 7-10 day intervals. A sample egg was collected from each nest for Persistent, Bioaccumulative and Toxic (PBT) contaminant analysis. A blood sample was also collected from a 40-45 day old nestling to assess exposure to pharmaceuticals and measure oxidative DNA damage as a general biomarker of toxicity. As in 2002, the percentage of eggs lost, eggs hatched and young fledged did not differ among regions. Productivity was within the range to maintain a stable population (0.8-1.15 fledglings/active nest). Nevertheless, ospreys appeared to have slightly lower productivity in the central Bay (1.11 fledglings/active nest in 2015 vs. 1.42 in 2002) and in the coastal Inland Bays (1.00 fledglings/active nest vs. 1.17 in 2002), while productivity appeared to increase slightly in the northern region (1.22 fledglings/active nest in 2015 vs. 1.00 in 2002). While the northern region exhibited 10.1% eggshell thinning in 2002, there were no differences across sites in 2015 ($p=0.39$; mean 0.50 ± 0.04 mm), with values approaching pre-DDT era thickness (0.505 mm). Two of 22 pharmaceuticals were detected in plasma. The analgesic, acetaminophen was present in 21 of 27 samples, with levels greater ($p=0.03$) in the urbanized northern region compared to the central region. The NSAID diclofenac was detected in 2 nestlings in the Inland Bays (< MDL-3.73 ng/mL). There was no evidence of genetic damage in blood among study regions ($p=0.61$). Our 2015 assessments of productivity, eggshell thickness, genetic damage and pharmaceuticals do not indicate substantial ecotoxicological risk for ospreys at the individual or population level. However, the status of fish-eating birds, and additional information on the overall environmental condition of Delaware Bay and River, will become clearer with results from the PBT contaminant analysis.

MP110 Increased rodenticide exposure rate and risk of toxicosis in barn owls from SW Canada – linkage with demographic not genetic factors

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Among many anthropogenic drivers of population decline, continual rapid urbanization and industrialization pose major challenges for the survival of wildlife species. Barn owls (Tyto alba) in southwestern British Columbia (BC) face a multitude of threats ranging from habitat fragmentation to vehicle strikes. They are also at risk from secondary poisoning of second-generation anticoagulant rodenticides (SGARs), a suite of toxic compounds which at high doses results in a depletion of blood clotting factors leading to internal bleeding and death. Here, using long-term data (N=119) for the hepatic residue levels of SGAR, we assessed the risk of toxicosis from SGAR for the BC barn owl population over the past two decades. We also investigated whether sensitivity to SGAR is associated with genetic factors, namely Single Nucleotide Polymorphisms (SNPs) found in the CYP2C45 gene of barn owls. We found that residue concentration for total SGAR was significantly higher in 2006-2013 (141ng/g) relative to 1992-2003 (57ng/g). The proportion of owls exposed to multiple SGAR types was also significantly higher in 2006-2013. Those measures accordingly translate directly into an increase in toxicosis risk level. We also detected demographic differences, where adult females showed on average lower concentration of total SGAR (64ng/g) when compared to adult males (106ng/g). Juveniles were overall more likely to show signs of toxicosis than adults (33.3% and 6.9%, respectively), and those symptoms were positively predicted by SGAR concentrations. We found no evidence that SNPs in the CYP2C45 gene of barn owls were associated with intraspecific variation in SGAR sensitivity. We recommend several preventative measures be taken to minimize wildlife exposure to SGAR.

MP111 Anticoagulant rodenticide residues in badger and fisher from southern British Columbia

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A number of studies from Europe and North America have reported relatively high incidence of liver residues of commonly used second generation anticoagulant rodenticide (SGAR) compounds, in mammalian predators. Some animals, primarily from California, also came from locations relatively remote from intensive urban or agricultural development. A proportion of animals tested have exhibited symptoms of anti-coagulant poisoning. For the present study we tested 40 samples of two species of mammal, the American badger Taxidea taxus, an endangered species in British Columbia, and a furbearer, the fisher Martes pennant, collected over the period 2004 to 2013 from locations in southern British Columbia, Canada. Of 30 badgers analyzed, 43% (13) contained residues of one of more AR. First generation, or FGARs, were the most commonly detected, wth warfarin and chlorphacinone predominant, but many animals had residues of both forms of AR. A total of 27% contained SGAR residues, most of which were > 0.1 µg/g with several > 0.5 µg/g. Bromadiolone was the most commonly detected SGAR but brodifacoum and difethialone were also regularly detected. Of 10 fisher tested, 60% (6) contained AR residues, five of which had SGARs, two in the range 0.4 to 0.6 µg/g. Spatial details will be presented along with an assessment of toxicological and conservation implications.

MP112 Investigating potential growth, behavioural, and reproductive effects of nestling exposure to methylmercury in Zebra Finches (Taeniopygia guttata)

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Methylmercury is a widespread contaminant that has been shown in multiple studies to cause behavioural and reproductive effects on piscivorous birds. It has been previously thought that birds that do not feed on fish (such as passerines) are not at risk for methylmercury toxicity. However, in recent years high blood mercury levels have been found in free living passerines. This has opened up a new area of study for methylmercury toxicity in avian species. Of the few previous studies on passerine species, most of them are lifetime exposure studies. Our lab's goal is to do shorter term exposures at different life stages using Zebra Finches (Taeniopygia guttata) as a model species to determine which stages are most sensitive. In the current study, the nestling stage was the target. Chicks hatched from clean (non-mercury exposed) parents were dosed with methylmercury (water only, 0.063 ug/ul, or 0.15 ug/ul per gram of body weight per day) from days 1 to 21 post hatch. This dosing period was used to simulate exposure from food provisioning by the parents. Growth of the chicks were measured until age 30, which is when they are considered independent. Despite a dose response relationship shown in our blood mercury analyses, no effects of dose were found for growth of the chicks. Once the chicks were over 90 days old (sexual maturity), mating trials with song recordings were conducted on the males while breeding experiments were conducted on the females. There were also no treatment effects found for male mating trials and song analyses or the female breeding trials. The lack of treatment effects in these experiments indicate that the nesting stage might be less sensitive in passerines, likely due the methylmercury burden being transferred to the growing feathers.

MP113 Pesticide transfer between fish and waterbirds of the West coast of Yucatan, Mexico

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Pesticide inputs in the Yucatan Peninsula have increased in the last years affecting coastal areas due to soil's karstic nature, which favors water to permeate through rock pores, cracks and solution conduits, causing the aquifer to be highly susceptible to the presence of pollutants. In this study, organochlorine (OCP) and organophosphate (OPP) pesticides were quantified in fish tissue (Gambusia yucatana and Poecilia velifera), as well as in alimentary bolus and plasma of three piscivorous waterbird species (snowy egret Egretta thula, great egret Ardea alba and roseate spoonbill Platalea ajaja) collected in Sisal and Celestun, located in the Western coastal area of Yucatan, Mexico. Pesticides were determined by ultrasonic assisted extraction, SPE (Florisil or C-18 6 mL/500 g cartridges), QuEChERS® clean-up and analytes were quantified by GC-MS. Total OCP concentrations in fish ranged from 131 to 948 ng/g dw and total OPP from 74 to 156 ng/g dw; no significant differences ($p< 0.05$) in pesticide levels were found between fish species and sampling sites. Samples of alimentary bolus were only obtained from A. alba (n=22) and E. thula (n=1) and pesticide content ranged from 48 to 708 ng/g dw for OCP and from 36 to 470 ng/g dw for OPP; statistically significant differences ($p< 0.05$) were found between sampling site. Minimum and maximum concentrations of OCP and OPP in plasma were 1 to 575 ng/mL and not detected to 127 ng/mL, respectively; significant differences in lindane, malathion and total OPP content in plasma were found and highest concentrations were detected in the roseate spoonbill (P. ajaja). Lindane, endosulfan, malathion, parathion and methyl parathion were detected in >85 % of the samples in fish tissue, bird alimentary bolus and bird plasma. Pesticides detected in fish and waterbirds suggest the transfer of these pollutans along food webs in the coast Yucatan; nevertheless, further studies are needed to evaluate if this transfer represents a risk for wild populations living in such ecosystems.

MP114 Experimental manipulation of dietary arsenic levels in great tit nestlings: effects on antioxidant molecules and lipid peroxidation

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The main objective of this study is to explore the potential effects of environmentally relevant arsenic (As) levels on oxidative stress biomarkers of great tits (Parus major) in Harjavalta, Finland. Nestlings were orally dosed with As in three experimental groups (Control, Low and High As groups: 0, 0.2 and 1 µg/g/d) and were compared with those living in the vicinity of a copper-nickel smelter, an As source (Smelter group, 0 µg/g/d). We studied a set of antioxidant molecules (glutathione peroxidase, GPx; glutathione-S-transferase, GST; catalase, CAT; superoxide dismutase, SOD; glutathione, GSH) and lipid peroxidation as TBARS (thiobarbituric acid reactive substances) levels. Feces were collected to measure As concentrations. Fecal As concentrations were significantly higher in the Smelter and High groups, followed by the Low group, and finally the Control group with significantly lowest levels. Although nestlings from the High As group showed higher GST and SOD activity and

GSH and TBARS levels than the Control group, no significant differences were found. CAT activity was significantly lower in the High As group, while GPx was significantly higher in the Smelter group when compared to the Control group. The lower CAT activity in the High As group may reflect the ability of this metalloid to deplete the activity of this antioxidant enzyme. The higher GPx activity in the polluted environment may reflect higher level of oxidative stress in nestlings from this area, probably due to the exposure to a mixture of metals and the associated limitation of resources (lower food quality and quantity) demonstrated before, showing the importance of secondary pollution effects on birds.

MP115 Effects of calcium supplementation and metal pollution on oxidative stress in great tit nestlings

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Different studies have suggested that calcium (Ca) administration may have a protective role against metal toxicity by modulating metal-induced oxidative stress. The present study aims to explore the effects of Ca availability and metal exposure on oxidative stress biomarkers in great tit (Parus major) nestlings. Great tits were supplemented with Ca (Ca-supplemented group) or not supplemented (Control group) during the breeding season in a metal-polluted and a control area in Harjavalta, Finland. Feces were collected to measure metal concentrations. We analyzed antioxidant enzymes (glutathione peroxidase, GPx; glutathione-S-transferase, GST; catalase, CAT and superoxide dismutase, SOD) and lipid peroxidation as thiobarbituric acid reactive substances (TBARS) levels in red blood cells of great tit nestlings. CAT activity was higher in Ca-supplemented nestlings, suggesting that Ca could be involved in the regulation of the antioxidant system by stimulating the activity of this enzyme. The activities of GPx and SOD were significantly higher in the polluted environment, which may reflect higher level of oxidative stress in nestlings from this area, probably due to the higher metal exposure and the poorer condition (nestling size and fledgling number were lower in the polluted area due to lower food quality and quantity). In this sense, GPx activity was positively correlated with metal concentrations in feces and negatively correlated with nestling size. Significant negative correlation was found between TBARS levels and nestling size, suggesting that smaller nestlings suffering from poorer condition have increased lipid peroxidation. However, the lack of differences in TBARS levels between the polluted and unpolluted zone shows that metal exposure seems to be low to directly produce oxidative damage to lipids.

MP116 Dynamic Modeling of Fish-Eating Bird First-Flush Exposure to Toxaphene in a Reservoir Constructed on Contaminated Farm Soil

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In 1990 EPA banned all uses of the last of the organochlorine (OC) insecticides, the highly persistent, bioaccumulative and toxic toxaphene, a mixture of polychlorinated camphenes. Subsequently, environmental scientists have been challenged to derive clean-up levels for toxaphene-contaminated farm soils for flooded land uses based on sound science rather than wishful thinking. Wishful thinking resulted in Florida's Lake Apopka disaster, where more than 500 fish-eating birds attracted to newly flooded wetlands on highly contaminated former farmland died from acute exposure to a mixture of rapidly bioaccumulating OCs in toxaphene-stunned fish in late 1998 and early 1999. Subsequently, the U.S. Fish and Wildlife Service adapted the Mackay Level III Fugacity Model to predict exposure of fish-eating birds bioaccumulating OCs from rapidly developing food chains in flooded depressions over OC hotspots to set clean-up levels. Partitioning of each OC is based on its measured or estimated soil/water partition coefficient normalized to organic carbon content (Koc) and subsequent bioaccumulation into each aquatic trophic level is based on its octanol/water partition coefficient (Kow). While the model appeared to reproduce observed OC bioaccumulation patterns when applied to the Lake Apopka wetlands, its deficiencies were obvious, as evidenced by greater bioaccumulation observed in the largemouth bass than the fathead minnow, despite an average lipid content of 3% and 6%, respectively. To correct USFWS model deficiencies, I developed a set of linked spreadsheets with sets of differential equations to model dynamic first-flush release of dissolved organic carbon and the OC from wet soils and subsequent OC dynamic bioaccumulation by a mudworm, and trophic level 1, 2, and 3 fish. The screening-level model was applied to toxaphene-contaminated Woerner Turf parcels in a proposed reservoir footprint in the Everglades Agricultural Area. The results suggest the USFWS model overestimates risks to fish-eating birds foraging in pools over low-lying toxaphene residue hotspots during first-flush flooding when fish at higher trophic levels do not reach steady state before the pools disappear with rising stage. Toxaphene fact sheets also need to be revised to remove erroneously low logKow values that may still be propagated into the derivation of contaminated site clean-up levels approved by responsible environmental protection and consulting wildlife protection agencies.

MP117 Blood and feather heavy metal concentration of a resident and migratory songbird

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Various anthropogenic activities have resulted in concentration of heavy metals and contamination of surrounding environments. Historically, heavy metal contamination at the Savannah River Site (SRS) in South Carolina has resulted from accidental releases of stored waste generated from nuclear weapon production in the early 1950's. Songbirds inhabiting and using resources from these areas have the potential to bioaccumulate metals but there is limited information on metal concentration levels in areas suspected of contamination as well as uncontaminated sites. Nonlethal tissue samples from avian blood and feathers provide a reliable approach for determining the bioavailability of these pollutants. The objective of this study was to survey bioavailable heavy metal contamination at the SRS though blood and feather samples from resident Northern Cardinals (Cardinalis cardinalis) and migratory Great Crested Flycatchers (Myiarchus crinitus). Samples were collected in April and May of 2016. Feather levels of chromium ($F_{1, 43}$ = 11.47, P =0.0015) and lead ($F_{1, 43}$ = 7.04, P = 0.009) were higher in flycatchers (Cr: =3.418 ppm, SD =3.888; Pb: = 0.291 ppm, SD =0.263) than cardinals (= 1.30 ppm Cr, SD =0.356; = 0.1436, SD =0.098). Selenium levels in blood were also significantly ($F_{1,34}$ = 9.83, P =0.004) higher in flycatchers (= 1.529, SD =0.355) than cardinals (= 1.067, SD =0.349). There was no difference in feather or blood concentrations between species for Hg, Ni, Zn, Cu, or Cd. Feather concentrations of Cu in male cardinals were more than females (B =1.119, 85% CL = 0.111, 2.128) in the greatest weighted model (w=0.533). The model with the greatest weight evaluating concentrations of Zn in the feathers of cardinals (w=0.999) indicated males have less than females (B=-46.186, 85% CL = -55.784, -36.588) and one location had less than the other sites (B=-38.198, 85% CL = -72.130,-4.267). Generally, both species had similar levels of all metals. Species differences are likely attributed to diet composition and time spent on their breeding grounds for flycatchers. Even though contaminant levels are below the toxicity thresholds, more research is needed to determine if these low levels of metal contamination can induce chronic stress and affect fitness or susceptibility to diseases and parasites.

MP118 Application of Relative Potency Data for Aroclor 1268 in Birds and Mammals

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Aroclor 1268 is an unusual PCB mixture containing primarily octa-, nona-, and decachlorobiphenyls. Several lines of evidence indicate that Aroclor 1268 is much less toxic than more typical environmental PCB mixtures. First, concentrations of the most potent (sometimes called "dioxin-like") PCB congeners in Aroclor 1268 are very low. Second, historical toxicity tests using chickens – a particularly sensitive species – indicated a lack of reproductive effects following exposure to Aroclor 1268, while comparable concentrations of other PCB mixtures caused significant reductions in hatching success. Third, a recent toxicity study using mink yielded dose-response relationships that show much lower toxicity compared to more typical PCB mixtures. While non-dioxin-like effects on mink were observed, they occurred only in association with notably high exposures. An Aroclor 1268 study in fish provides additional supporting information, although data on PCB effects of Aroclor 1268 versus other PCB mixtures are from different fish species. Taken together, these lines of evidence support the derivation of relative potency factors to interpret Aroclor 1268 exposures in birds and mammals, relative to toxicity data developed for other PCB mixtures. We provide a case study evaluating PCBs in least terns and bottlenose dolphins residing near an Aroclor 1268-contaminated site in coastal Georgia. Although elevated total PCB concentrations in these species initially prompted concerns regarding potential reproductive effects, such concerns are not justified when the relative potency of Aroclor 1268 is taken into account.

MP119 The Effects of Sub-Lethal Pesticide Exposure on Behavior of Aquatic Vertebrates: A Meta-Analysis

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Pesticide contaminants are ubiquitous in aquatic environments and pose a threat to biodiversity. Behavioral indicators, commonly used in ecological and toxicological studies to evaluate sub-lethal effects of pesticides on aquatic organisms, create important connections between physiological and ecological processes. Evaluating behavioral shifts in response to exposure can integrate concepts of ecology with the field of toxicology to improve how we evaluate the impacts of pesticide exposure. In order to bridge the toxicological and behavioral literature, and identify chemical classes that denote the largest threat, we conducted a meta-analysis summarizing the effects of pesticides on swim speed and activity of aquatic vertebrates. We found that exposure to environmentally relevant concentrations of pesticides reduced the swim speed of exposed amphibians and fish by 49 percent, and reduced overall activity by 71 percent. There were also differences in the magnitude of this effect across chemical classes, which likely reflect underlying physiological processes. Pyrethroids, carbamates, organophosphates, and triazines all produced a large decrease in swim speed, where as phosphonoglycines and organotins showed no overall effect. Pyrethroids, carbamates, organophosphates, organochlorines, and organotins also produced a large decrease in activity, while phosphonoglycines had no overall effect, and triazines had the opposite effect of increasing activity. Our results indicate that even sub-lethal concentrations of pesticides have strong effects on critical behaviors that affect fitness and alter species interactions in aquatic vertebrate species. We expect our synthesis can be used to identify chemical classes producing the largest sub-lethal effects for further research and management.

MP120 In situ Method for Evaluating the Early Life Toxicity of Contaminated Sediments in Walleye (Sander vitreus)

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Early life exposure to environmental contaminants can cause persistent effects in fish that are only manifested later in life. In the wild, it is difficult to study the effects of early life exposures since it can be challenging to find fish spawning grounds and it's extremely difficult to track these developing fish once they have hatched. Exposures can be done in the laboratory, but these experiments do not accurately reflect environmental conditions. Here we assess the feasibility of an in situ exposure of developing fish embryos to clean and contaminated sediments in the Saint-Lawrence River. Eyed walleye (Sander vitreus) eggs were placed in Scotty incubators and deployed at two sites (May 2016) in the Saint-Lawrence upstream of Montreal, where the river widens to form Lac Saint-Louis. Sediments at the contaminated site (Beauharnois) are known to be heavily contaminated with PAHs, metals, PCBs, dioxins, and furans. This contamination is most likely due to discharges from the industrial areas and commercial ship traffic nearby. The reference site (Sainte-Anne-de-Bellevue) is located on the western side of Lake Saint-Louis. The walleye egg incubators were placed in protective cages, which were lined with fine mesh, before being completely submerged and anchored at the study sites. After the walleye eggs hatched, larvae swam out of the incubators escape holes and were collected in the protective cage and preserved for molecular analyses. Traditional (e.g. EROD, oxidative stress) and novel (e.g. DNA methylation) biomarkers of exposure to environmental contaminants were measured in larvae from the two sites. In addition, sediment and water samples were collected from each site and were analyzed for metals and polycyclic aromatic hydrocarbons (PAHs). The use of this in situ method, in conjunction with more controlled in vitro and in vivo laboratory-based dosing studies that we have undertaken, will be beneficial for assessing the impact that early life exposure to contaminated sediments has on the health of wild populations of fish.

MP121 Toxicity of silver nanoparticles in biosolid-amended soil to Eisenia fetida: perspectives for the protection of agricultural soils and the environment

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The growing number of commercial applications using silver nanoparticles (Ag-NP) increases the risk of environmental release and exposure of organisms in natural environments. Land application of biosolids is accepted practice in Canada and viewed as positive for agricultural soils to boost fertility, improve soil structure and enhance soil biological activity for the benefit of crops. Biosolids enriched with Ag-NP may pose an ecological risk to soil organisms and plants, but this is poorly understood. The objective of this experiment was to investigate the impact of Ag-NP and Ag-nitrate ($Ag\text{-}NO_3$) on earthworms Eisenia fetida. Controlled laboratory experiments were conducted with artificial OECD soil and a natural agricultural soil (Brown Chernozem) that were amended with 0 to 1000 mg of Ag-NPs and $Ag\text{-}NO_3$ per kilogram of soil. Earthworms were exposed to Ag-NP in vivo for 28 days and a range of endpoints was assessed, including survival, growth, reproduction and histopathology. In addition, Ag-NP treated soil samples were analysed to determine Ag-NP transformations, including dissolution to Ag^+ in soil pore water with single-particle ICP-MS. Results will be used to propose specific toxicity mechanisms of Ag-NP to the earthworm E. fetida, specifically whether toxic effects were the result of exposure to Ag-NP or dissolved Ag^+. Perspectives on applying biosolids containing Ag-NP as a soil amendment, in the context of sustainable agriculture and environmental protection, will be discussed.

Integrating Life Cycle, Risk and Alternatives Assessment with Exposure Modeling for Chemical Decision-Making

MP122 Chemical Exposure Footprint: Application and Evaluation of the ProScale method in an indoor furniture case study

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The case study presented is performed as part of IVLs participation in the ProScale consortium, developing a method for estimating hazard and exposure potentials for chemicals in products, in a life cycle perspective. The primary intended use of the method is as an exposure/hazard potential scoring to be provided as additional information in LCA based product performance assessments. The study aims to contributing to the evaluation of the relevance, accuracy and robustness of the ProScale method by applying the draft ProScale method, in a case study covering the Life cycle of a product, and testing alternative approaches in each step of the method, i.e. release/exposure/hazard potential. The chosen product is a sofa, as an example of a product for indoor use, containing a variety of materials and with potential for relevant chemical exposures and hazards to estimate, in the use phase as well as upstream in the production value chain and downstream in the end of life (waste management) phase. Ultimately all substances in all life cycle phases should be covered, but we started by looking at a specific chemical, Melamin, CAS No 108-78-1, as previous work has shown that this substance is common as flame retardant additive in natural rubber latex foam, and has a relatively high release rate in this use, and thus exposure potential. Other substances of interest are, e.g. TCPP, CAS no 13674-84-5, also used as flame retardant in this type of product. A major challenge in this project has been to identify the specific composition of a specific product, therefore a more population level exposure approach has been tested. For each relevant substance in the life cycle, the value chain has been identified. Then for each life cycle phase, the exposure has been characterized using, to the extent possible, predefined exposure categories from REACH, "PROCs". This allows for exposure potential estimate using the dedicated tool ECETOC TRA. For consumer exposure during use also other approaches have been tested. These exposure potentials have been combined with the hazard potency factor, as described in another submission to this conference about ProScale. The presentation will display preliminary result regarding hot spots in the life cycle of the sofa, in terms of the chemical exposure footprint

Novel Mechanisms of Nanomaterial Toxicity Through Direct Exposure or Indirect Interactions with Environmental Components

MP123 Determination of Carbon Nanotube Phytotoxicity in Heat Stressed Crops

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Climate change, which is currently of great concern to the science community and general public, may lead to dramatic changes in weather patterns. These changes dictate a better understanding of how ecosystems might be affected, including effects on crop growth as well as the fate and toxicity of contaminants. Carbon nanotubes (CNTs) are currently used in a wide-range of commercial and agricultural applications. The increasing use of CNTs in fertilizer, pesticide, and herbicide delivery systems could have direct, unintended consequences on soil health, the microbiome, and managed and native ecosystems. Limited studies have examined the impact of CNTs on abiotic stress response and potential consequences on plant CNT uptake with respect to plant performance and subsequent potential human exposure to CNTs. In this study, the effect of CNTs in soil on plant performance under periodic thermal stress (heat waves) was examined. Cotton and tomato were grown in soil spiked with multi-walled carbon nanotubes (MWCNTs) (100 mg/kg soil) under two temperature regimes with natural lighting: 1) Control T = 28/18°C day/night and 2) Elevated T = 32/22°C day/night. The impact of elevated growth temperature and the interaction with MWCNTs on water use efficiency (WUE) was measured, along with the impact of periodic heat waves on photosynthesis. Preliminary analysis of this data indicates that the presence of MWCNTs in soil significantly decreased WUE in cotton, but not in tomato. Exposure of cotton plants to MWCNTs under heat stress had a negative effect on plant growth and photosynthetic rate. These results may add a new variable to the equation for CNT effects on plants. Past studies have shown that results vary depending upon plant species and CNT type. Climate may exacerbate the impact of CNTs on plant growth.

MP124 Modulation of Immune Defense Responses of Fathead Minnows exposed to Single-Walled Carbon Nanotubes

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Single walled Carbon Nanotubes (SWCNTs) are being used in the production of a number of industrial and commercial products yet little is known about the potential environmental and human health impacts of these materials. Previous work in our lab has shown that SWCNTs can alter the activity of toll-like receptors (TLRs), proteins that act as a first line of defense against invading pathogens. Our lab has also observed, in mammalian systems, that increased viral titers occur with pre-exposure of cells of mice to SWCNTs. To date, no studies have investigated whether SWCNTs can alter viral infectivity in fish through modulation of normal immune defense responses that include TLRs. To begin to address this question, fathead minnows (Pimephales promelas) were fed a commercial diet containing SWCNTs, for 96 hours and the levels of the viral responsive TLR3 was measured by qRT-PCR in various sections of intestinal tissue (proximal, middle, distal). Data from these experiments show that TLR3 expression was significantly increased in distal intestinal tissues while no change in expression occurred in proximal and middle sections. To determine if the SWCNTs altered viral susceptibility, we exposed fish to SWCNTs for 7 days followed by a second exposure to Polyinosinic:Polycytidylic acid (Poly I:C), an RNA analog that is a TLR3 agonist used to mimic viral infection. Following exposures, fish were euthanized and blood was analyzed for changes in white blood cell (WBC) populations. Preliminary results show altered WBC populations (neutrophils) that are consistent with viral infections. Currently we are investigating how the SWCNTs may be altering this response and the associated mechanisms that contribute to these effects. These studies highlight a novel sub-lethal mechanism of toxicity that should be considered for nanomaterials.

MP125 Overcoming Carbon Nanomaterial Interference in qPCR Assessments of Gene Expression Changes in Exposure Experiments

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Interference in standard assays by nanomaterials (NM) is commonly observed and can lead to inaccurate or misinterpreted results. There are a number of reasons that account for such interference including altered absorbance and fluorescence readings and chemical reactivity with and sorption of assay reagents. Through recent experiments investigating gene expression changes in human lung cells exposed to NMs we found that specific carbon nanotubes impaired at least one step in the qRT-PCR pipeline. Our initial results showed that amplification of target and reference genes was altered in a NM dose-dependent manner. The average quantification cycles (Cq) for the reference gene GAPDH in control and pristine single-walled carbon nanotube (SWNT) exposed samples were consistent at 15.8 and 15.5 cycles, respectively. However, samples exposed to 20 µg/mL surface-hydroxylated multi-walled carbon nanotubes (MWNT) exhibited delayed amplification presenting with an average Cq of 20.6 cycles. To pinpoint whether the interference was occurring during RNA quantitation, reverse transcription (RT) or PCR steps we used a multiple quantitation strategies, PCR and RT inhibition curves. These experiments showed that the greatest interference occurred during the RT step. Since we only observed interference with functionalized MWCNTs we hypothesized that these select NMs were adsorbing vital RT reagents and limiting cDNA synthesis. To improve the RT process we increased the amount of available reagents by doubling the reaction volume. A second strategy was to add BSA (0.4 µg/µL) to reduce the available binding sites thereby limiting reagent adsorption by MWNTs. Results revealed that both strategies improved the cDNA synthesis. For example, doubling the RT reaction volume stabilized GAPDH Cq values between controls and 20 µg/mL MWNT treated samples, decreasing the Cq difference to 1.5 cycles while addition of BSA was more effective and decreased the difference to 0.69 cycles. These studies highlight the need to run proper interference controls when working with NMs. Data further support the notion that not all NMs impact assay interference equally and in fact, select properties, such as surface functionalization, are important determinants to assay variability and accuracy. Determining mechanisms by which different NMs interfere with standard assays will allow for better assay designs to limit interference and produce high quality results.

MP126 Global gene expression profiling in zebrafish (Danio rerio) exposed to nanoparticles of maghemite (y-Fe2O3)

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Iron oxide nanoparticles (IONPs) have been used for several biomedical applications, such as contrast agent for magnetic resonance imaging. However, the potential toxicity of various IONPs in the environment is not well understood yet. To gain insights into the toxic mode of action of IONPs, a gene expression analysis was performed on adult male zebrafish exposed to 96 h treatment with maghemite NPs (average diameter of 0.5 µm) at 37.2 mg/L and 74.4 mg/L, compared to a control group (without treatment). The transcriptome profile (Affymetrix GeneChip Zebrafish Gene 1.0 ST Array) was obtained from hepatic RNA and the data analyzed using R. Gene Ontology analysis was conducted with the DAVID bioinformatics resources 6.7. Microarray analysis showed significant changes in expression of 953 genes (ANOVA, $p < 0.05$). Hierarchical clustering of the significantly changed genes separated control samples from the group treated with 74.4 mg/L maghemite NPs, whereas samples treated with 37.2 mg/L maghemite NPs falled somewhere in between. Maghemite NPs induced changes in a concentration-dependent manner. Partitioning Around Medoids algorithm established two as the optimal number of clusters to classify the 953-gene dataset. Cluster 1 (380 genes) included genes whose mRNA abundance increased with maghemite NPs exposure, whereas genes included in Cluster 2 (573 genes) showed a parallel decrease in maghemite NP-treated animals relative to control. A clear dose-response was observed for most genes included in both clusters, consistently with the hierarchical clustering data. Functional analysis of genes included in clusters 1 and 2 shows several cellular functions affected by the exposure to maghemite NPs. Among the genes up-regulated by NPs there is an overrepresentation of functions related to cation/metal binding and epithelium and embryo development. These genes include several typical stress proteins, like HSPB1. We consider that this cluster reflects the physiological response to the presence of the iron NPs and some associated distortion with the cation balance in the cells. Conversely, genes whose expression decreases upon maghemite NPs exposure shows an enrichment of translation- or ribosome-related genes, suggesting a decrease on the ability of the cell to produce new proteins. We propose that these changes indicate an inhibitory effect on cell metabolism. Our results shed light on the molecular mechanisms of liver toxicity of IONPs.

MP127 Investigation of the influence of carbon nanotubes on the bioavailability of mixtures of PAHs to P. promelas

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Due to the high adsorption affinity of carbon nanotubes (CNTs) for organic contaminants, these materials are often suggested as an ideal substrate for pollution remediation. CNTs significantly reduce the bioavailability of a range of polycyclic aromatic hydrocarbons (PAHs) in single solute exposures to a variety of organisms. It is important to note though, that in both waste water treatment facilities and in the environment, CNTs will interact with more than one organic contaminant resulting in adsorption competition among the compounds present. Studies have found that adsorption capacity and affinity for individual PAHs in mixtures can be significantly altered due to competition and interaction among the PAHs at CNT adsorption sites. To date, no study has investigated the bioavailability of mixtures of PAHs adsorbed to CNTs though this is a much more likely scenario to occur environmentally and may elicit different trends in bioavailability than previously observed in single-solute scenarios. The goal of the present research was to investigate the adsorption behavior of PAH mixtures on CNTs and how this relates to the resulting bioavailability of the PAHs present. Bi-solute adsorption isotherms of a suite of PAHs to CNTs were established in conjunction with quantifying the bioavailability of the two competing CNT-adsorbed PAHs to Pimephales promelas (fathead minnow) using bile analysis via fluorescence spectroscopy. Ratio comparison of the fish response to each PAH present in treatments with and without CNTs provide insight to the interaction occurring between the PAHs at the CNT surface. Previously established results indicate that bioavailability of CNT-adsorbed PAHs is a function of adsorption behavior and the PAHs physical characteristics, where molecular configuration and size were found to be significantly influential. Preliminary data suggest that when in mixtures, linear PAHs outcompete the angular isoform for adsorption sites on CNTs causing angular PAHs to be more bioavailable. This is attributed to differences in adsorption capacity of CNTs for chemically similar yet structurally different PAHs. In addition, data suggests that in mixtures, PAHs containing a non-aromatic ring, such as FLU, are more bioavailable than fully aromatic PAHs due to fully aromatic PAHs adsorbing more strongly. Results of this work will help to provide insight into the effectiveness of CNTs to reduce PAH bioavailability in more realistic scenarios.

MP128 Novel toxicity associated with emerging hybrid nanomaterials in aquatic systems

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Engineered nanomaterials (NMs) represent a diverse class of extremely small particles (< 100nm) that are being widely used in industrial sectors such as energy, electronics, and sensing. As these applications demanded higher performance, the field of materials research has shifted its focus from using singular NMs (carbon only, metal only) to those comprised of several distinct types of NMs linked together, termed nanohybrids (NHs). Linking single NMs in this capacity is likely to create new properties and behavior in environmental and biological settings that have not yet been predicted or studied. We hypothesize that NHs alter fish growth and bioenergetics differently than individual nanoparticle constituents and that these effects are consistent with novel NH properties such as morphology, mechanical stiffness, and band gap. To test this notion, we synthesized a set of metal-carbon nanotube NHs (MWCNT + TiO_2 + ZnO) with high degree of control and exposed fathead minnows aqueously to the NHs and singular components following a standard EPA 96 hour toxicity testing regime. Fish were monitored for mortality and growth. These endpoints were coupled with high-throughput contemporary measurements of mitochondrial dysfunction and oxidative stress. We also characterized physical morphology, mechanical stiffness, band gap and distribution of metal/metal oxides on nanotubes and compared these measurements between NHs and individual component materials. Preliminary data show that carbon nanotube-metal NHs produce distinct effects on fish growth and mitochondrial function and that these effects are coupled to unique material properties. This work is timely in that it will lay the foundation for further research in understanding NHs in complex but relevant environments and reveal novel properties and mechanisms of action in biological systems that have not been studied. Furthermore, the work will generate critical and fundamental knowledge to better understand the environmental interaction of a set of complex hierarchical nanomaterials.

MP129 In vitro to in vivo extrapolation of nano titanium dioxide cytotoxicity in Zebrafish (Danio rerio)

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Titanium dioxide nanoparticles ($nTiO_2$) are one of the most studied nanoparticles in the toxicology literature, as well as being one of the most heavily utilized nanoparticles today. Uses include but are far from limited to cosmetics, sunscreens, solar energy production, and in surface coatings with self-cleaning and biofouling resistant properties. Nanoparticle enhanced products whose end use places them outside (e.g. surface coatings) have the potential to emit nanoparticles directly into surface waters without filtration through storm water runoff. Although $nTiO_2$ is among the more thoroughly studied nanoparticles, a recent Web of Science search on the topic of “nanoparticle” with either “TiO2” or “titanium dioxide” in the article title and refined to “toxicology” suggested there are relatively few fish studies (112) on $nTiO_2$ compared to mammalian in vivo and in vitro studies (345) in the literature. The majority of the fish studies only use in vivo or in vitro, but do not investigate both. Among fish model organisms the zebrafish (Danio rerio) was chosen for its extensive in vivo use in existing non-nanotoxicological literature, ease of culture, and the availability of a well established cell line. Zebrafish hepatocytes (ATCC CRL-2643) were exposed to a range (0.001 µg/mL to 10.0 µg/mL) of $nTiO_2$ to assess cytotoxic response using the resazurin metabolism assay. Experiments using a factorial design are on-going to investigate the effects of sera on $nTiO_2$ interaction with cells. Hepatocytes will be exposed to the determined EC_{50} in several treatments varying the amount of sera present in the exposure medium (0% fetal bovine serum ,0% trout serum to 10% fetal bovine serum, 5% trout serum). Cellular uptake of $nTiO_2$ will be qualified by TXRF and quantified by ICP-MS. $nTiO_2$ partitioning within the cell will be investigated by subcellular fractioning and quantification of titanium within the fraction. Oxidative stress indicators (e.g. TBARS, SOD, Catalase) and stress proteins (e.g. hsp 70, HIF-1α) will be used as endpoints. In vitro model results will be compared to in vivo exposure of D. rerio embryos to similar concentrations of $nTiO_2$. As with the in vitro investigations, uptake will be quantified, and partitioning in subcellular fractions will be determined. These studies are expected to narrow the gap between $nTiO_2$ in vitro models and in vivo observations in teleost fish.

MP130 Investigation of Toxicity of Perovskite Nanomaterials (a new solar cell nanomaterial) in Zebrafish and Linkage to Release of Pb2+

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Next generation solar cells incorporate perovskite-based nanomaterials (NMs) that demonstrate high stability and efficient capture of solar energy for the generation of electricity. While the attributes of perovskite nanomaterials are being realised, any potential consequences of the technology, including their toxicology upon release into the environment, must be assessed. Perovskite materials have the ABX_3 crystal structure and as a standard they are methyl ammonium lead halides. The chemical reactions of perovskite materials in the aqueous phase can lead to release of Pb^{2+} and consequent toxicity in aquatic organisms. The aim of this research is to assess the toxicity of two perovskite nanomaterials ($CH_3NH_3PbI_3$ and $CH_3NHNH_3PbBr_3$) in zebrafish larvae and the linkage to Pb^{2+} toxicity. A dilution series of the two perovskite NMs for acute toxicity tests (96-h exposure) and metallothionein 2 (mt2) expression (24-h exposure) were prepared in parallel with Pb^{2+} dilution series. The 96-h median lethal concentration (LC_{50}) for both perovskites was greater than 200 mg/L in zebrafish larvae (exposure from age 72 hours post fertilization), indicating no acute toxicity. Positive mt2 expression was observed in relationship with Pb^{2+} (as lead nitrate) concentration after a 24-h exposure (R^2=0.91). Mt2 expression increased with perovskite NMs higher concentration (0 to 80 mg/L of Pb^{2+} that perovskite particles contain according to molecular weight) in a positive dose-response relationship, and was consistent with Pb^{2+} (as lead nitrate) dilution series (0 to 80 mg/L). Determination of mt2 gene expression suggests that much of the toxicity can be attributed to the presence of Pb^{2+} in the perovskite NMs.

MP131 PAHs Sorption to NMs in the Aqueous Phase Assessed by Evaluation of Bioavailability in Larval Zebrafish

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Engineered nanoparticles (NPs) have unique physicochemistry that includes high surface area, surface reactivity, and photoactivity. In the aqueous phase, NPs can sorb substances and subsequently undergo complex chemical reactions that alter the chemistry of sorbed substances. Understanding NP-substance sorption is an important consideration for managing the environmental risks of NPs. Among the most commonly used NPs are TiO_2-NPs which have photo-activity and have been shown to sorb various substances in the aqueous phase, including toxic substances. Polycyclic aromatic hydrocarbons (PAHs) are a group of important environmental toxicants that can have UV-enhanced toxicity and have potential to sorb to NPs. The aim of this research was to investigate the sorption of PAHs to NPs under UVA illumination. In particular, the objectives of this study were to assess the phototoxicity of TiO_2-NPs, Si-NPs, and PAHs (anthracene and benzo(a)pyrene), sorption of PAHs to NPs and how this association affects bioavailability and toxicity in larval zebrafish. This was addressed by evaluating the sorption of PAHs to NPs by assessment of changes in expression of target biomarker genes including cytochrome P450 1A (cyp1A), superoxide dismutase (sod1) and DNA repair (ddb2), in larval zebrafish, following exposure to these substances and particles with and without UVA irradiance. Zebrafish larvae (age 72 hpf) were exposed (24 h) to anthracene (0-30 µg/L) in synthesised freshwater (OECD 236). Preparations were exposed to 80 kJ/m^2 UVA at the end of the 24 h exposure, and larvae were sampled for extraction of RNA and RT q PCR. Photo-activated anthracene increased cyp1A expression in a positive dose response relationship while when NPs were present in solution, no induction of the gene was observed. Expression of cyp1A increased with concentration of benzo(a)pyrene (no UVA exposure). No changes in cyp1A expression were observed when TiO_2 NM105 or Si-NPs were present in the dilution series. In contrast, when a smaller TiO_2 particle (4-8 nm dimeter) was added in the dilution series, the expression of cyp1A was significantly reduced (GLM, p< 0.001), suggesting that sorption processes are surface area dependent. Future work will investigate the association between NMs and benzo(a)pyrene after UVA irradiance to assess whether the UVA photoactivation of the particle surface occurs and leads to changes in sorption processes.

MP132 Effects of ingestion of antimicrobial nanoparticles on endogenous microbiota and pathogen resistance in rainbow trout

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Incorporation of engineered nanoparticles (NPs) into consumer products is increasing rapidly and contamination of the environment with potential exposure of organisms has become a realistic concern. Ingestion of NPs by organisms is an environmentally relevant exposure route and effects on gut health and physiology are important. The antimicrobial activity of some NPs (e.g. Ag- and Cu-NPs), which is useful in medical and personal care products, could have negative effects on beneficial/protective bacteria within the digestive system of organisms. The importance of healthy endogenous gut microbiota on overall organism health is increasingly recognized and has important implications on immune system function and resistance to pathogens. Our objective was to investigate effects of ingestion of food containing NPs (Ag-NPs, Cu-NPs) or bulk material controls ($AgNO_3$, $CuSO_4$) on gut microbial community, immune system function, and pathogen resistance in juvenile rainbow trout (Oncorhynchus mykiss). Fish were exposed to 50 mg metal (as NP or bulk material) per kg feed (or control feed) for 56 days and sampled for assessment of gut microbial community (by Illumina 16s rRNA gene amplicon sequencing and analysis in MG-RAST), gut health (gene expression and histopathology), and immune system function (hematology, gene expression). After the exposure, fish were challenged with the bacterial pathogen Yersinia ruckeri, the causative agent of enteric redmouth (ERM) disease in rainbow trout. We found that dietary exposure to Ag-NP and Cu-NP (or bulk controls) did not affect growth or survival, but initial evaluation of gut microbiota suggest that feed treatments do change the abundance of various bacterial genera. Within one week of exposure, fish displayed clinical signs of ERM disease (external lesions) and mortality was 57 (9-100) %, but there was no significant difference among feed conditions. Associations between changes in gut microbiota, immune system function, and pathogen infection are currently under investigation.

MP133 Enhanced and differential genotoxicity of titanium dioxide nanoparticles by expression of human CYP genes in yeast cells

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Titanium dioxide nanoparticles ($nTiO_2$) are increasingly being manufactured with extensive applications in a wide range of fields. Due to their unique physicochemical properties including high redox potential and large surface-area to mass ratio, $nTiO_2$ may exert an adverse health impacts. Although $nTiO_2$ have been classified as possible carcinogens to humans, data on their genotoxicity remains limited and controversial. The production of reactive oxygen species has been postulated to play a role in $nTiO_2$ genotoxicity. However, most existing studies have not considered the role of cellular metabolism activity in assessing the genotoxicity of $nTiO_2$. Here, a toxicogenomics-based in vitro genotoxicity assay using GFP-tagged yeast reporter strains, covering key protein biomarkers indicative of all the recognized DNA damage repair pathways, was employed to elucidate the genotoxic potential and mechanisms of $nTiO_2$. In addition to the original yeast reporter library, two GFP-tagged yeast reporter strain libraries transformed with human CYP1A1 or CYP1A2 gene were constructed to investigate the potential modulation of $nTiO_2$ genotoxicity after P450 metabolism. The genotoxicant 4-nitroquinoline-1-oxide and a non-genotoxic agent tetracycline were used as positive and negative controls, respectively, to validate the assays. Then the genotoxicity of CYP1A1-dependent chemical benzo[a]pyrene-7,8-dihydrodiol and CYP1A2-dependent chemical aflatoxin B_1 as well as $nTiO_2$, were assessed in three reporter libraries, the first with no CYP expression, the second expressing CYP1A1 and the third expressing CYP1A2. These studies found a lack of appreciable $nTiO_2$ genotoxicity as indicated by the GFP-tagged reporter strains in the absence of CYP metabolism and a significantly elevated indications of genotoxicity after activation by both CYP1A1 and CYP1A2 enzymes ($p = 0.0075$ and 0.0032 respectively). The intracellular ROS production measurement indicates significantly higher ROS ($p < 0.05$) in yeast expressing either the CYP1A1 or CYP1A2 enzymes (2.17 and 3.03 times higher respectively). We also detected mitochondrial DNA damage suggesting mitochondria as likely one of the target sites for oxidative damage by $nTiO_2$ after metabolic activation by both human CYP enzymes. These data thus indicate that the genotoxicity of $nTiO_2$ is enhanced by human CYP1A1 and CYP1A2 expression and is associated with elevated oxidative stress, and suggest that similar toxicity could occur in human cells.

MP134 Evaluation of the toxicity of newly synthesized titanium dioxide nanoparticles

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Titanium is one of the most commonly used metals for cosmetic products, fabrics, dye and other household industry. The production, number and significance of titanium based nanomaterials (NPs) such as TiO_2 are growing very fast, but information about toxic effects and interactions of the TiO_2NPs with living systems is contradictory in the literature. Understanding the impacts of nanomaterial exposure in ecosystems is important to appropriately protect the environment from increased NP pollution, particularly in aquatic systems. Therefore, we evaluated toxicity of newly synthesized, differently-doped or core-shell NPs using Xenopus laevis and Danio rerio embryos. Toxicity of NPs was also evaluated in Chlamydomonas reinhartii, Daphnia magna neonates and using inhibition of bioluminescens of Vibrio fischeri. Our results showed that S- or Mn-doped TiO_2NPs did not cause significant lethality on X. laevis and D. rerio embryos after 96 hours up to 500 mg/L NP exposure. However, D. magna neonates were adversely affected by both materials after 48h exposure in higher concentrations. Toxicity of Mn-doped TiO_2 was higher than S-doped TiO_2 to daphnids. We found that none of the core-shell NPs elicited a concentration-related lethality or malformations to frog or zebrafish embryos up to 250 mg/L exposure. Tested NPs did not cause growth inhibition of X. laevis embryos or algae after 96 h of exposure at any concentration. Microtox tests with V. fischeri supported our results that no toxic effect was found for any of the NPs tested. While none of newly synthesized TiO_2 based NPs were acutely toxic to the aquatic organisms tested here, the interaction of photocatalytically active NPs with organic pollutants may lead to secondary toxicity effects from redox alterations of the chemical or TiO_2NPs. Acknowledgement: This bilateral collaborative project was supported by grants from The Scientific and Technological Research Council of Turkey (TÜBITAK) (Project #113Z561) and National Science Foundation (NSF) in The USA (Project #1438165).

MP135 Identification of Biomarkers in Corn Subsequent Silver Nanoparticles Exposure

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Nanomaterials are increasingly used in agriculture, energy production, and biomedical application due to their unique properties and characteristics. There is limited information available regarding their exact chemical makeup, raising concerns for their impact on the environment and human health. Many of the nanomaterials contain metals and one of the most popular nanoparticles contain silver, which is known to be toxic in its ionized form. Silver nanoparticles (AgNPs), have a core-shell silver structure and are used in many facets of the industry, including: clothing, food packaging, cosmetics, and electronic products, mostly due to their antimicrobial properties. Due to their wide range of applications, AgNPs containing materials are disposed of in land fills, introducing a pathway for AgNPs contamination in the environment. In addition, AgNPs can leach out from products during washing, reach wastewater treatment plants, and accumulate in biosolids. Consequently, the land-application of biosolids to fertilize fields or usage of reclaimed water can lead to the unintentional exposure of AgNPs to crops. The goal of our project is to understand the impact of AgNPs on hydroponically grown corn with emphasis on monitoring the molecular response of the organism to AgNPs exposure. Specifically, the corn's root systems are investigated, as they are the route for initial exposure. Corn plants were exposed to AgNPs, and changes in hydrophilic metabolite composition of the roots were determined using liquid chromatography tandem quadrupole time of flight mass spectrometry. Additionally, silver concentrations were determined in corn leaves and roots via inductively coupled plasma mass spectrometry to determine the fate of AgNPs in corn. Evaluating the impact of changes in metabolite composition will be critical for identification of biomarkers of AgNP exposure. Preliminary results have shown a clear difference between treatments, the identification of species that differentiate during AgNP treatment are to be determined. Correlating the silver concentration in corn and changes in metabolites will provide insights on the mechanism and interactions between nanomaterials and this agricultural crop. Investigating the effects that AgNPs have on food crops is paramount for understanding the potential toxicity and the metabolic changes associated with AgNPs contamination.

MP136 Toxicological evaluation and photocatalytic degradation capability of newly synthesized nanoparticles

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Due to recent developments in nanomaterial engineering technology, TiO_2 nanoparticles (NPs) are being used for core-shell NP particle production for efficient photodetection capability to effectively remediate organic pollutants in aqueous environments. However, limited information is available in the literature to evaluate potential risks of the materials and single-species toxicity tests are inadequate to predict the effects of chemicals in an ecosystem. Therefore we compared the toxic effects of newly synthesized TiO_2 NPs using the African clawed frog (Xenopus laevis) and zebrafish (Danio rerio) embryos in a 96 h toxicity test for comparison of their toxicity. To this aim, the toxic effects of pure TiO_2 and core-shell TiO_2 (SiO_2@TiO_2) NPs were evaluated. On the other hand, the photocatalytic degradation capability of the NPs were evaluated using disperse red 65 dye, which is in widespread use, highly toxic and a potential pollutant for aquatic ecosystems. According to 96 h subacute toxicity tests, none of the NPs represented toxicity potential on test organisms and they did not cause any developmental malformations on X. laevis and D. rerio embryos. On the other hand, the LC50s of disperse red 65 were determined as 4.67 mg/L (4.40-4.99) and 1.74 mg/L (1.56-1.96) for frog and zebrafish embryos, respectively, in 96 h toxicity tests. Due to the high toxicity potential of the dye, 20 mg/L (4-11X higher concentration of LC50s) concentrations were treated with 0.3% catalysts for up to 4 h in optimal conditions. According to our results, the tested dye caused different types of malformations on both test organisms. After the photocatalytic degradation, pure TiO_2 NPs enabled up to 94% degradation of the dye within the 1 h exposure period and SiO_2@TiO_2 NPs degraded 88.32% of the dye within 3 h. The lethality effect on X. laevis embryos was completely dissipated after 3 h of photodegradation of the dye. However, although lethal effects were relatively decreased for zebrafish embryos, they were not completely removed. Pure TiO_2 NPs were also most successful for decreasing the lethal effect after 4 h photocatalysis compared to SiO_2@TiO_2 core@shell structure. This may be related to total effective mass and total surface area of pure and core@shell NPs for photocatalytic capability. Acknowledgement: This project was funded by The Scientific and Technological Research Council of Turkey (TÜBITAK) (Project #113Z561).

MP137 Form and toxicity of copper released into marine systems from conventionally and nano-sized copper treated lumber

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The fate and effects of pristine engineered nanomaterials (ENMs) in simplified systems have been widely studied; however, little is known about the potential release and impact of ENMs from consumer goods, especially lumber that has been treated with micronized copper. Micronized copper solutions contain copper complexes predominately in the 10-700 nm size range, and are used in lumber to prevent microbial degradation and fouling. In this work, the goal was to determine the rate, concentration, and form of copper released from commercially available pressure treated lumber samples (blocks and sawdust) exposed to an aqueous system. Lumber tested included Southern Yellow Pine (SYP) treated with micronized copper azole (MCA) at 0.96 and 2.4 Kg/m^3, alkaline copper quaternary (ACQ) at 0.30 and 9.6 Kg/m^3, and chromated copper arsenate (CCA) at 40 Kg/m^3. The experimental system included wood cubes cut from the outer 2 cm surface of the lumber or the equivalent mass (4 g) of sawdust submerged in 250 mL of media (0, 1, 10, and 30 ppt filtered natural seawater) in polyethylene bottles, and mixed on a shaker table at 120 rpm. Water samples were taken at 8 hours, and on days 1, 2, 7, 14, and 28 for the blocks and days 1, 2, 3, 7, 17, and 28 for the sawdust. Subsamples included unfiltered water (defined as 0.45 µm – filtered water for the sawdust), and water filtered through a 0.1 µm polyethersulfone (PES) syringe filter, and a 3000 Dalton centrifugal filter, which were analyzed using ICP-AES to determine the total, nano+ionic copper (< 0.1 µm subsample), and approaching ionic copper (Americamysis bahia) were used to confirm the results of the size-based analyses for the 0 and 30 ppt treatments, respectively. Overall these results suggest that the form of copper released from treated lumber is ionic and that little nanocopper is present in the aqueous phase. This finding suggests the risk associated with nanocopper treated lumber can be addressed with existing copper ion-based modeling approaches and regulations.

MP138 Environmental fate and transport of titanium dioxide nanoparticles in municipal wastewater effluent in outdoor stream mesocosms

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Titanium dioxide (TiO_2) nanomaterials are ever more widely used for many applications and across many industries. As their production and use continues to increase so does the risk of release into aquatic environments. However, risk assessments for engineered nanomaterials have been challenging. While there is a growing body of research on the toxicity of TiO_2 nanoparticles (NPs), risk assessments must also account for TiO_2 NP behavior, fate and transport, and bioavailability at environmentally relevant concentrations in complex ecosystems. Here, we present fate and transport results of a 35-day stream mesocosm study. TiO_2 NPs were added continuously for 28 days into 20 m long artificial streams running partially recirculating municipal wastewater effluent to reach nominal TiO_2 concentrations of either 5 mg L^{-1} (high) or 50 µg L^{-1} (low), in addition to control streams with only effluent. Results from ICP-MS analyses for Ti concentrations in water indicate that the low streams remained similar to the controls, ranging from 1-8 µg L^{-1}, indicating that the low concentration was environmentally relevant, especially for effluent-dominated waters. On the other hand, the high streams had concentrations that remained relatively stable throughout the dosing period at 100-300 µg L^{-1}. There was up to an 80% decrease of Ti in the water column 1 day after cessation of dosing in both the low and high streams, followed by a less dramatic steady decrease in the final week of the study. Periphyton concentrations in control and the low streams similarly did not differentiate from each other, with concentrations of 100-600 mg Ti kg^{-1} dry weight, with no observed accumulation or increase in Ti concentration throughout the 28 day exposure period. However, periphyton Ti concentrations in high streams increased throughout the first 28 days, reaching concentrations of 2,500-10,800 mg Ti kg^{-1} dry weight. A 40% decrease in Ti concentration was observed at day 35, 7 days after cessation of Ti treatment. The riffle, run and pool habitat types had differing concentrations of Ti in periphyton as a result of location relative to the dosing site and/or flow conditions and water depth. Periphyton in the run section with fish grazing activity had lower concentrations of Ti than in the ungrazed sections, indicating that both stream region and level of disturbance influenced Ti concentrations in periphyton.

MP139 Phytoplankton taxonomic community dynamics during a whole lake nanosilver addition

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Due to its increasing use as an antimicrobial, many recent studies have examined the impacts of nanosilver on aquatic taxa. In short term studies, phytoplankton have been found to have a higher tolerance to nanosilver than other trophic levels such as zooplankton and bacteria. However, effects of nanosilver on phytoplankton communities over the longer term and under more natural conditions have not yet been tested. We performed a whole lake nanosilver addition experiment at the Experimental Lakes Area in which we added 15 kg of nanosilver to a lake over two consecutive ice-free seasons. Phytoplankton communities and silver concentrations were monitored in the study lake and a reference lake for two years prior to and two years during additions to monitor whether any changes occurred. Nanosilver accumulated in the phytoplankton community and ranged between 0.2-4.5 µg Ag/mg C during additions. This amount accounted for up to one fifth of the total silver in the water column. Seasonal and annual variation in the composition of phytoplankton biomass was apparent, but nanosilver had little effect on the overall phytoplankton biomass or taxonomic composition, despite accumulation in the phytoplankton community. Succession in phytoplankton community composition in the treatment lake did not differ from that of the reference lake and both were highly synchronous. Overall, we demonstrate that nanosilver at environmentally relevant concentrations is not particularly harmful to phytoplankton. Because nanosilver binds to phytoplankton it could buffer the impacts of nanosilver to other parts of the ecosystem.

MP140 An Ecosystem Scale Experiment: Fate and Effects of Silver Nanoparticles Following Whole-lake Addition at the Experimental Lakes Area

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The potential release of nanoparticles into aquatic environments is raising global concerns. As antimicrobials, silver nanoparticles (AgNPs) are among the most prominent nanoparticles in use. Despite this, their fate, long-term toxicity, and ecological relevance have yet to be investigated under natural settings. To better understand environmental significance, we released AgNPs (NanoAmor 30-50 nm PVP capped) into a lake at the Experimental Lakes Area. We added a total of 9 kg in 2014 and 6 kg in 2015 using a dosing system set on the shore of the lake to simulate a point source addition. Overall, AgNPs remained suspended in the water column and were detected throughout the lake and in the lower food web. Mean total Ag increased to up to 7.5 µg/L in 2014 and 17.5 µg/L in 2015 at the center buoy location. Total Ag concentrations were highly dynamic seasonally both in the epilimnion and hypolimnion depending on the physical, chemical and biological patterns of the lake. Single particle ICPMS analysis revealed 90.7% of the particles had a diameter that was less than 100 nm in August 2014 suggesting large agglomerates were infrequent in the water column. Ag accumulated in all lower food web components. At the center buoy location Ag accumulated in the bacterioplankton up to 1.4 µg/L, in phytoplankton up to 3.5 µg/L, in zooplankton up to 56 µg Ag/g dry mass, and in macroinvertebrates up to 26 µg Ag/g dry mass. At the end of the second year of addition, we found concentrations of Ag in the lake sediments up to 4.73 µg Ag/g dry mass, indicating AgNP settling from the water column. Despite AgNP being measured throughout the lake, we found no effects on the lake food web. For example, chlorophyll concentrations varied similarly between the exposed and reference lakes and leaf-litter decomposition rates were similar between the exposed lake and other reference lakes at the ELA. The presence of dissolved and particulate organic matter likely reduced toxicity. Our experiment provides the first whole-lake perspective toward AgNP fate and toxicity, suggesting small scale experiments may overestimate environmental responses.

MP141 Mechanisms for toxicity of a next generation energy storage nanomaterial to Daphnia magna

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While previous studies have investigated the behavior of simple metal oxides, emerging technologies such as energy storage are utilizing more complex and multi-component nanoscale metal oxides consisting of layered structures and redox-active metals. Energy storage materials may be made of a wide range of transition metals depending on application. Furthermore, these materials may be substituted for others to achieve different properties or to increase environmental compatibility. First-generation materials such as lithium cobalt oxide (LCO) are being replaced by next-generation nanomaterials such as lithium nickel manganese cobalt oxide (NMC). Next-generation electric vehicles are predicted to utilize more than 100 kg of nanoscale Li-NMC per vehicle as a battery cathode material, leading to many potential exposure scenarios. In this study, we are comparing LCO to NMC as a model system for assessing the potential environmental impact of nanomaterials for energy storage and to determine if metals within these materials may be substituted to mitigate environmental impact. We chose Daphnia magna as a surrogate for assessing the environmental impact of these materials. The impacts of these particles on D. magna were determined in acute and chronic assays, measuring endpoints such as body size, reproduction and mortality. Acute studies demonstrated no effect on daphnid mortality up to 25 mg/L whereas chronic studies show significant impacts to daphnid reproduction at the lowest concentration tested, 0.25 mg/L. Lithium cobalt oxide caused similar impacts to reproduction and survival at concentrations four times less than NMC nanoparticles. Toxicity from metal dissolution was accounted for and showed no effect, leading us to believe that this is a nanomaterial-specific impact either through direct contact and localization of metal ions or through a nanomaterial-specific impact on physiology. Lastly, our study investigated gene expression. Results indicate down-regulation of a number of important genes related to metabolism in nanomaterial exposures that did not correlate with exposures to the background dissolved metals in solution. This study shows that we may be able to mitigate negative biological impacts by altering battery material chemical composition.

MP142 Effects of natural suspended matter on toxicity of silver nanoparticles to Daphnia Magna

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Silver nanoparticles (AgNPs) have been widely used in many commercial products, such as appliances and antibacterial products. Thus, there are increasing concerns for the potential environmental risks of AgNPs to aquatic ecosystems. However, various environmental variables impact the toxicological effects of AgNPs to aquatic organisms. In addition, the influence of various natural suspended particles on the behavior and toxicity of nanoparticles has rarely been studies. Therefore, the purpose of this study was to elucidate the impact of natural suspended matters on the toxicity of silver compounds including AgNPs and $AgNO_3$ to Daphnia magna. To determine LC50 values, D. magna were exposed either to a range in concentration of $AgNO_3$ or AgNPs in artificial hard water under static conditions for up to 48h. The median lethal concentration (LC50) of AgNPs and $AgNO_3$ to D. magna were 22 µg/L and 1.8 µg/L, respectively. After 48 hours of exposure to $AgNO_3$ and AgNPs, D. magna mortality showed an inverse relationship with concentration of suspended particles including algae and seston. Thereafter, $1x10^4$ ~ $1x10^6$ cells/ml of a phytoplankton Selenastrum capricornutum or 50 ~ 250 mg/L of montmorillonite were suspended either in 4 µg/L of $AgNO_3$ or in 40 µg/L of AgNPs. The mortality of D. magna in $AgNO_3$ medium was significantly reduced from 100% to < 2% with an increase of suspended particles; either by increasing the algal concentration from $1x10^4$ cells/ml to $1x10^6$ cells/mlor by increasing montmorillonite concentration from 100 mg/L to 250 mg/L. Similarly, the mortality in AgNPs treatments was significantly decreased from 100% to < 15% either by increasing montmorillonite concentration from 50 mg/L to 150 mg/L or by increasing algal concentration from $1x10^4$ cells/mlto $1x10^6$ cells/ml. The reduction of mortality was accompanied with reduction in dissolved Ag^+ concentrations in the exposure media; dissolved Ag^+ concentration decreased with increasing time and suspended particle concentration. In particular, the Ag^+ concentrations were below a detection limit at the highest suspended particle concentrations after 48h exposure in the both media. Our study clearly demonstrates the significant role of natural suspended particles in reducing the toxicity of silver compounds, which was largely explained by a reduction in dissolved Ag^+ concentration.

MP143 Mediation of Metal Toxicity to Meiobenthic Copepods by Polyvinylpyrrolidone Coating of Silver Nanoparticles

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As commercial and industrial uses for silver nanoparticles increase – principally as biocides – environmental releases through sanitary waste discharge and degradation of Ag-nano containing products are growing. Most silver-induced toxicity in aquatic systems is from metal dissolution to free Ag-ion, although more generally there is a residual effect which cannot be explained by free ion alone. The capping of Ag nanoparticles (Ag-nanos) by organic polymers such as polyvinylpyrrolidone (Ag-PVP) has been proposed as a means to stabilize Ag-nanos in solution. The possibility of Ag-nano toxicity reduction via particle coating was explored using Ag-PVP as a model particle and full-lifecycle comparative exposures of copepods to seawater-dissolved reference $AgNO_3$ (0, 20, 30, 45, 75 ug · L^{-1}) versus treatment PVP coated Ag-nanos (same concentrations) using the meiobenthic copepod Amphiascus tenuiremis per ASTM E2317-04(2012) methods. Over the full lifecycle, $AgNO_3$ dissolved in 30S seawater at only 30 ug · L^{-1} was significantly lethal to copepods (45% of test population dead in 26 days) with mating success cut in half and fecundity reduced 40%. In contrast, Ag-PVP at 30 ug · L^{-1} produced only 19% population death by 26 days with no negative effects on mating success or fecundity. Response data for stage-specific survival rates, sex ratios, mating success and fecundity were modeled via Leslie (Lefkovich) matrices to project an integrated population response model for these two forms of seawater-associated Ag. PVP-coated particles produced a population doubling time of 3-4 generations across all test concentrations except the control (i.e., 2 generation doubling time). $AgNO_3$ at 20 ug · L^{-1} pushed population doubling-time to 4 generations versus 2; and > 30 ug · L^{-1} pushed copepod populations to extinction in 9-12 generations. Ag nanoparticle coating by PVP appears to slow particle dissolution and thus confer population-relevant protection from the stronger toxicity observed for rapidly-dissolving $AgNO_3$ in seawater.

MP144 Cellular Specific Responses of the Eastern Oyster, Crassostrea virginica, to PolyDOTs

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Polymer dynamic organic theranostic spheres (PolyDOTs) are currently being studied as a potential to be used in photothermal ablation treatment of cancer. If shown to be successful, these PolyDOTs will be mass-produced and it is expected that they, like any other engineered nanoparticle (ENP), will be found in the ambient environment. The specific PolyDOTs used in these studies are P3HT/BSe PolyDOTs and are activated to thermally ablate cancer cells by 808nm of near infrared light. In order to investigate the potential of even greater toxicity (e.g. potentiation) due to solar exposure, which includes UV radiation, infrared radiation, and longer wavelengths of light, the PolyDOTs were exposed to the sun for 12 hours and effectively "charged". Once charged, hepatopancreas and gill tissues from the eastern oyster (Crassostrea virginica) were exposed to various concentrations of both uncharged and charged PolyDOTs for 24 hours, and the effects of the PolyDOTs on lysosomal destabilization were analyzed using the Neutral Red assay, cell viability was analyzed using the MTT assay, and free radical damage was analyzed using the lipid peroxidation assay. Preliminary data from the lysosomal toxicity assays with in vitro hepatopancreas tissues exposed to PolyDOT particles showed that there were increased rates of lysosomal destabilization in charged exposures compared to uncharged. The MTT toxicity assay showed that there were no statistically significant differences between the exposure groups when tissues were exposed to both charged and uncharged PolyDOTs. Studies like these are important for understanding the effects of ENPs on oysters, and are also important for understanding the broader ecological impacts of ENPs on other species and estuarine ecosystems.

MP145 Accumulation of dissolved silver and silver nanoparticles in liposomes used as a model membrane

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The increasing presence of nanomaterials in consumer products has led the scientific community to study the environmental fate of these contaminants of emerging concern. Understanding their transformations and interactions with living organisms is a crucial step in the study of their impacts on aquatic ecosystems. Silver nanoparticles, used mainly for their antibacterial properties, are among the most common nanomaterials. How they interact with aquatic organisms, especially how they cross the biological membrane, remains uncertain. In this project, we are studying the uptake of dissolved silver and silver nanoparticles by liposomes. These unilamellar vesicles composed of phospholipids have long been used to model natural biological membranes. This allows studying the potential uptake of silver by passive diffusion through the phospholipid bilayer. The liposomes were synthesized in a pH 6 buffer using extrusion techniques and potential membrane leakage was monitored throughout. Size exclusion chromatography was used to remove the outer buffer and the liposomes were then exposed over time to silver under different conditions where Ag^+, $AgS_2O_3^-$ or $AgCl^0$ were the dominating species. At the end of the exposure, Ambersep GT74 cation exchange resin (0.3 g per sample, > 95% efficient) was used in order to bind the non-assimilated dissolved metal. Similar experiments were conducted with the complexes $HgCl_2$

and $Cd(DDC)_2$, both hydrophobic and known to diffuse passively through the biological membranes. Finally, liposomes will also be put in contact with 5-nm PVP-coated silver nanoparticles. For these experiments, a second size exclusion chromatography step will be used to separate silver nanoparticles and exposed liposomes. The uptake kinetics of Ag^+, $HgCl_2$ and $Cd(DDC)_2$ show no increase over time, unlike $AgS_2O_3^-$ and $AgCl^0$, which appear to go through the phospholipid bilayer. This seems in contradiction with our initial hypothesis that lipophilic Hg and Cd complexes would be able to cross the membrane while silver would not.

MP146 Interaction of Nanoparticles with Model Bacterial Surfaces

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Bacteria are ubiquitous in terrestrial and aquatic environments and perform critical functions within these ecosystems. The interaction of bacteria with nanomaterials released into such environments may elicit adverse effects or result in nanoparticle transformation. One of the primary modes of nanomaterial interaction with bacteria involves contact with the bacterial cell surface. Our objective was to determine the critical chemical components of Gram-negative and Gram-positive bacterial cell surfaces that govern nanoparticle interaction. To study these interactions, we constructed model Gram-negative bacterial cell surfaces containing lipopolysaccharides and isolated Gram-positive peptidoglycan with covalently bound wall teichoic acids (anionic glycopolymers). We used cationic nanoparticles as a model system for nanoparticles with an inert core and positively charged surface. We employed complementary spectroscopic, imaging, and in situ microgravimetric techniques to study the interaction of these nanoparticles with model bacterial cell surfaces. We found that the extent of nanoparticle attachment to model Gram-negative cell surfaces is dictated by concentration and type of lipopolysaccharides. Preliminary solid-state NMR results suggest that positively charged diamond nanoparticles associate with wall teichoic acids. We expect that our results will ultimately inform the assessment of risk posed by nanomaterials released into the environment, as well as nanoparticle manufacturing to modulate interactions with Gram-negative and Gram-positive bacteria.

MP147 Aggregation, sedimentation, dissolution and bioavailability of quantum dots in estuarine systems

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Due to increasing use in flat screen applications, solar cells, ink–jet printing, and medical devices, cadmium-based quantum dots (QDs) are among the fastest growing classes of engineered nanomaterial. These wide-ranging consumer product applications and end of use disposal issues assure that QDs will eventually enter the marine environment. In order to understand the fate and transport of CdSe QDs in estuarine systems, the aggregation, sedimentation, dissolution, and bioavailability of CdSe QDs in seawater was investigated. The size of CdSe QDs increased from 40-60 nm to >1 mm within one hr once introduced to seawater, and the diffusion-limited aggregation led to highly polydispersed aggregates with loose structures. As a result, the sedimentation rate of CdSe QD aggregates in seawater was measured as 4-10 mm/day, which was slow considering their relatively large size. Humic acid (HA), as a model natural organic matter, further increased the size and polydispersity of CdSe QDs, and slowed their sedimentation. Natural sunlight and light filters were employed to simulate the photic conditions at different water depths in an estuarine system. It was observed that light played a vital role in promoting the dissolution of CdSe QDs and the release of dissolved Cd. The ZnS shell surrounding the CdSe core also significantly hindered the degradation of CdSe QDs into their ionic components. With sufficient light, the presence of HA increased the dissolution of QDs, while with relatively scarce light, HA alone did not significantly change the dissolution of QDs. Our results demonstrate that the benthic zone in marine systems is the most probable long-term destination of CdSe QDs due to aggregation and sedimentation, despite being affected by slower transport processes than expected. In addition, the benthic community might be exposed to both particulate and ionic forms of CdSe QDs. The bioavailability of QDs to epibenthic organisms was evaluated by using mysid Americamysis bahia as a model organism, and the 7-day LC50s of particulate QDs and dissolved Cd was 290 and 23 ppt, respectively. For this organism, the acute toxicity appears to be largely a result of dissolved Cd from the QDs; however, research on the toxic effects of particulate QDs should be conducted with other animal models where QDs may be lodged in critical tissues such as gills or filtering apparatus, and Cd ions may be released and delivered more directly to those tissues.

MP148 Nanotoxicology of NMC, PAH-AuNP, Citrate-AuNP and D. melanogaster

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Due to its mapped, highly tractable genome, high percentage of homologous disease genes to humans, and a short ~10 day life cycle, D. melanogaster is an exceptional model for examining potential toxic effects of exposure to NMC and other nanoparticles. Investigations of the potential environmental impacts of nanoparticles have largely focused on model or simple nanomaterials. The current work exposes Drosophila melanogaster to poly(allylamine) hydrochloride wrapped gold nanoparticles (PAH–AuNPs), and citrate capped gold nanoparticles (Cit–AuNPs) as well as first generation battery materials lithium cobalt oxide ($LiCoO_2$) and complex next-generation battery material lithium nickel manganese cobalt oxide (NMC). This work represents the first steps towards evaluating potential toxic effects of battery-related nanoparticles on D. melanogaster. In initial trials, no significant mortality was observed in D. melanogaster larvae exposed to NMC nanoparticles at 1, 10, 20 or 100 mg/L, utilizing two different methods: nanoparticle-treated food media and nanoparticle-treated aqueous sucrose solution. Previously published studies with Drosophila commonly exposelarvae to nanoparticles via mixture with food media, which introduces additional variables that may affect outcomes. Nanoparticles are less stable when introduced to food media, and homogenous mixing is difficult; and the methods described in existing research are often vague, e.g. nanoparticles were "mixed strongly" into the food media. The work presented here describes a minimal media exposure method in which D. melanogaster embryos hatch directly into treatment media consisting of only nanoparticles and moderately hard water. Results seen in D. melanogaster will be used to compare impacts across various model organisms.

MP149 The Interactive Effects of UV Radiation and Titanium Dioxide Nanoparticles on the Calanoid Copepod, Acartia tonsa

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Titanium dioxide nanoparticles (TiO_2 NP) are used in a variety of consumer goods including paints, sunscreens, skin-care products and anti-microbial agents. As a result of their prevalent use, especially related to beach and boating activities, TiO_2 NP are likely entering the marine environment via direct and indirect inputs. Nanoscale TiO_2 NP are photocatalysts, able to produce reactive oxygen species (ROS) that can damage living cells and cause deleterious effects in some aquatic organisms. This study investigated the effects of commercially available TiO_2 NP on the female calanoid copepod, Acartia tonsa. Four experimental treatments, a) copepods with NP and food, b) copepods with food only, c) NP with food and d) food only, were exposed to three different light conditions (15-hr light, 9-hr light and 0-hr light as a control). Light was supplied by solar lamps that mimic natural light in the visible and UVA/B regions.

An oceanographic optical model, Hydrolight, was used to determine the appropriate environmental light levels for experiments. Initial and final algae concentrations were determined to evaluate the effects on copepod ingestion rate (IR). Egg production rate (EPR) was determined after 24 hours of exposure, and egg hatching frequency (HF) was determined at 48 hours. Preliminary results indicate that there is a both NP and light effect on copepod IR, EPR and HF, compared to the NP and 0-hr light controls. These and other effects will be presented. This work suggests that NP under realistic environmental conditions could have possible organismal and population impacts, and will help to inform future studies on organisms that rely on zooplankton for food (e.g., fish larvae and filter-feeders).

MP150 The Comparative Study of Silver Nanoparticles and Ionic Silver Exposure on Organ Pathology of Sprague-Dawley Rats

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Silver nanoparticles (AgNPs) have potent physical, chemical and anti-bacterial properties, which has lead to their application in a wide variety of industrial and medical products. This study investigated the toxicity, distribution, and accumulation of AgNPs in the digestive tract, liver, spleen, brain, and bone tissue of female Sprague-Dawley rats. In addition, the effect of Ag ions was also examined. The experimental specimens were exposed to AgNPs synthesized using cetyltrimethyl ammonium bromide (CTAB) as a stabilizer through the reduction of silver nitrate ($AgNO_3$) with sodium borohydride ($NaBH_4$) in water. The solutions were administered orally in water for an 18-day period. The following treatments were established: colloidal AgNP 20 ppm daily, silver ions (Ag^+) from silver nitrate ($AgNO_3$). The following controls were prepared: CTAB control with a concentration of 1mM and tap water. Experimental behavioral observations were conducted. At the termination of the experiment, the rats were sacrificed by CO_2 overdose and organs were collected for further analysis. Throughout the experimental exposure, the rats receiving the AgNPs became visibly more lethargic, their fur coat lost its luster, and the animals shed their fur in tufts. Significant time difference between treatments was observed during the euthanasia process. The rats exposed to the AgNPs treatment died in approximately 20-30 sec, 1 min in $AgNO_3$ rats, 2 min in CTAB rats, while in the tap water control the lethal exposure was about 3-4 minutes. Rats in AgNPs treatment had visible hepatomegaly and splenomegaly. They also had much thinner and more brittle veins that snapped upon touching. The gross observations suggest that the administration of AgNP's orally had an adverse effect on the health and systematic functions of Sprague-Dawley rats.

MP151 Nanoparticles feeding exposure to the marine amphipod Parhyale hawaiensis: absorption in hemolymph

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Silver nanoparticles (AgNP) tend to agglomerate in the aqueous phase and settle to sediment surfaces where they present risk of toxicity to deposit feeding organisms. Amphipods, like Parhyale hawaiensis, are a key deposit-feeding species and an excellent model for evaluation of AgNP toxicology. We hypothesized that exposure of P. hawaiensis via ingestion of food contaminated with AgNPs would lead to absorption of Ag into internal tissues and toxicity. The first task was to establish a method for measuring silver concentration in the hemolymph of these very small organisms (e.g., P. hawaiensis has 5 to 10 mm length when adult). Silver nanoparticles < 100nm (Sigma Aldrich) or elemental Ag (from AgCl, Sigma Aldrich) were incorporated (500 mg kg^{-1}) into formulated fish feed pellets as described in Merrifield et al (2013). Each adult organism was placed individually into a plastic container (100 mL of reconstituted saline water), and fed daily with control, AgNP, or AgCl amended feed pellets. After 1 hour, each organism, was washed and placed into a new plastic container with clean salt water to ensure that the exposure was only via food. After 4, 7 and 14 days of exposure, hemolymph (approximately 0.5 µL per organism) was collected. An Agilent 7700x Inductively Coupled Plasma Mass Spectroscopy was used for Ag determinations. The results showed that a higher amount of silver was absorbed from AgNP feed than silver from AgCl in all exposure times. In the case of AgNP feed, increase of silver concentration was related to exposure duration. From chemical analysis of the hemolymph, it appeared that ingested silver is more bioavailable to as AgNP than when it is salt in form (AgCl). Microscopy of organisms is underway to evaluate if the nanoparticles are absorbed as particles or Ag ions. The ability to analyze metals concentrations in the hemolymph of P. hawaiensis extends the importance and utility of this organism as a model for ecotoxicology.

MP152 Exploring impacts of complex nanomaterials using the nematode C. elegans

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The nematode C. elegans is an extremely well-studied model organism and represents a powerful tool for elucidating mechanisms of toxicity of nanomaterials. Nanotoxicology exposures have to-date focused largely on simple materials such as silver, but manufactured nanomaterials with potential environmental impacts are likely to be complex. The work presented here represents the initial stages in the exploration of the interactions of complex nanomaterials using the powerful model organism, C. elegans. Adult nematodes were exposed to the complex next-generation battery material Lithium Nickel Magnesium Cobalt Oxide (NMC) as well as negatively and positively-charged gold nanoparticles: citrate-capped gold (Cit-Au) and poly(allylamine) hydrochloride-wrapped gold (PAH-Au) spheres. Nanomaterials were mixed with OP50 bacteria and spread on solid NGM plates, simulating a sediment-type exposure. Animals were observed and scored alive/dead daily until the end of their lifespan. In contrast to results observed in aquatic exposures with animal models such as Daphnia magna; NMC, Cit-Au, and PAH-Au did not cause increased mortality in C. elegans, even at concentrations as high as 100, 10, and 10 mg/L respectively. These results suggest that media may play an important role in toxicity of nanomaterials, but represent only the beginning of explorations of complex nanomaterial interactions using C. elegans. The well-studied and amenable nature of this organism make possible future studies taking advantage of gene-expression, gene-knockdown and knockouts, GFP-reporters, and myriad types of microscopy.

Assessing Risks of Pesticides to Federally Listed (Threatened and Endangered) Species at a National Level – Part 2

MP153 A Refined Aquatic Ecological Risk Assessment for the California Tiger Salamander Potentially Exposed to Malathion

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The California tiger salamander (CTS; Ambystoma californiense) is endemic to the California grasslands. CTS is federally threatened throughout California and endangered in Santa Barbara and Sonoma Counties. Under the Federal Insecticide, Fungicide and Rodenticide Act, the United States Environmental Protection Agency (USEPA) registers pesticides for use in the US. Registration or reregistration of a pesticide is the authorization of a "federal action" under the Endangered Species Act. As such, USEPA is required to ensure that pesticide registration actions are unlikely to jeopardize threatened and endangered species or their critical habitat. In 2010, USEPA issued its malathion effects determination for the CTS. Based on its conservative and deterministic risk assessment, USEPA made a Likely to Adversely Affect determination for the CTS. After the release of the National Academy of Sciences panel report (NAS, 2013), the USEPA, Fish & Wildlife Service, National Marine Fisheries Service and the US Department of Agriculture adopted an interim approach for conducting endangered species assessments for pesticides. The Interagency Interim Approaches consist of three steps: (1) a conservative analysis to determine which listed species could be exposed, (2) refined analyses to determine risks to individuals, and (3) population modeling to determine if species are in "jeopardy" from pesticide use. The Interagency Interim Approaches are currently being tested with three case study pesticides including chlorpyrifos, diazinon and malathion. Draft Biological Evaluations for these three chemicals were released by USEPA in April 2016. In light of the current interim approaches and the need for improved risk estimation, a refined assessment was carried out and focused on direct and indirect effects to the CTS. A custom approach to modeling malathion exposures in surface waters inhabited by CTS was developed for the refined assessment. This approach consisted of using the Pesticide Root Zone Model and Variable Volume Water Model along with input parameters customized to reflect soils, slopes, weather, crop and pond geometries observed in CTS habitat range. We also used a weight-of-evidence approach and evaluated independent lines of evidence (e.g., field and mesocosm studies, monitoring data and incident reports). Based on the results of the weight-of-evidence assessment, currently labeled uses of malathion are unlikely to result in direct or indirect effects to CTS.

MP154 A Refined Ecological Risk Assessment for the Delta Smelt Potentially Exposed to Malathion in California

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The Delta smelt (Hypomesus transpacificus) is an annual fish endemic to the Sacramento-San Joaquin Estuary. The species was listed as threatened on March 5, 1993 by the United States Fish and Wildlife Service (US FWS). Under the Federal Insecticide, Fungicide and Rodenticide Act, the US Environmental Protection Agency (USEPA) registers pesticides for use in the US. Registration or reregistration of a pesticide is the authorization of a "federal action" under the Endangered Species Act. As such, USEPA is required to ensure that pesticide registration actions are unlikely to jeopardize threatened and endangered species or their critical habitat. In 2010, USEPA issued its malathion effects determination for Delta smelt (DS). Based on its conservative and deterministic risk assessment, USEPA made a Likely to Adversely Affect determination for the DS. After the release of the National Academy of Sciences panel report (NAS, 2013), the USEPA, FWS, National Marine Fisheries Service and the United States Department of Agriculture adopted an interim approach for conducting endangered species assessments for pesticides. The Interagency Interim Approaches consist of three steps: (1) a conservative analysis to determine which listed species could be exposed, (2) refined analyses to determine risks to individuals, and (3) population modeling to determine if species are in "jeopardy" from pesticide use. The Interagency Interim Approaches are currently being tested with three case study pesticides including chlorpyrifos, diazinon and malathion. Draft Biological evaluations for these three chemicals were released by USEPA in April 2016. In light of the current interim approaches and the need for improved risk estimation, a refined assessment was undertaken and focused on direct and indirect effects to the DS. The Soil and Water Assessment Tool (SWAT) was used to predict aquatic EECs in the water bodies within the DS critical habitat. The application of the SWAT model considered all uses of malathion within the watershed draining into the California Delta, including the Sacramento and San Joaquin drainages. We also used a weight-of-evidence approach and evaluated independent lines of evidence (e.g., field and mesocosm studies, monitoring data and incident reports). Based on the results of the weight-of-evidence assessment, currently labeled uses of malathion are not expected to result in direct effects to DS and would rarely affect its aquatic invertebrate prey.

MP155 Refined Aquatic Exposure Estimates based on Species Data from the Pilot Biological Evaluations

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The draft biological evaluations (BEs) released by USEPA assessing three pilot chemicals utilized a regionalized assessment methodology for assessing exposure to endangered species living in aquatic environments. Aquatic exposure was assessed using several fixed receiving water (habitat) dimensions, referred to as aquatic bins. The BEs also included a detailed summary of USFWS information regarding habitat waterbodies and species locations. In this presentation, a tiered exposure assessment methodology is proposed that builds off of the scenario based exposure methodologies, but also extends beyond and utilizes species relevant data as early in the process as feasible. Refinements addressing habitat hydrodynamics with appropriate modeling approaches will be presented as refinements using readily available modeling tools and conservative assumptions. In the presentation of results, USEPA clearly identified extreme exposure predictions and expressed desire for ideas on refinements. This presentation seeks to answer that call in a species relevant and specific manner that allows for scaling to the appropriate chemical use footprint with focus on species habitat area. Results allow for use in screening level deterministic risk assessments. They can be further integrated with surrogate species dose response curves or sensitivity distributions for a species focused probabilistic risk expression. Temporal analysis could also be derived from the results. Implementing a species focused analysis framework using actual habitat data to inform model refinements also provides the opportunity to incorporate mitigating factors in the exposure assessment. Model tools should incorporate naturally occurring landscape mitigating factors (i.e. landscape based treatment buffers from listed stream habitats, percent crop area) as well as label or management based mitigation measures (i.e. drift buffers or protected lands). The goal of a national assessment should be to generate relevant exposure estimates by including best available data in a pragmatic way.

From Source to Sink: Consumer Product Chemical Exposures and Exposure Pathways

MP156 Assessing Risk for Consumer Products Under California's Proposition 65 Regulations

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Proposition 65, formally known as the Safe Drinking Water and Toxic Enforcement Act of 1986, is a California initiative that requires businesses to warn consumers if exposures to listed chemicals exceed their chemical-specific Safe Harbor Levels. Over 900 chemicals potential carcinogens and reproductive toxicants spanning various industries such as food, drugs, consumer products, cosmetics, etc. are listed and the list is continually being updated. It is critical that businesses planning on selling their products in California ensure that their products are Proposition 65-compliant. However, demonstrating compliance may be convoluted because in many cases Safe Harbor Levels have yet to be developed (e.g., recently added bisphenol-A[BPA]); due to the type of user involved (e.g., consumer or worker); types of chemicals involved (e.g., carcinogen or reproductive toxicant); and the varying routes of exposure (e.g., dermal, inhalation and/

or ingestion routes). Here, we provide case studies of different approaches on conducting Proposition 65 risk assessments using real world examples of consumer and worker products using different listed chemicals. Cases discussed include: 1) inhalation exposure to salon workers from formaldehyde in hair products; 2) dermal and ingestion exposure to children from diethyl hexyl phthalate (DEHP) and BPA in bath toy products; and 3) dermal exposure to consumers from nickel (Ni) in wearable products. These case studies show the diversity of approaches that are used to assess risk from everyday use of common consumer products.

MP157 Environmental Obesogens: An Emerging Concern

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Human exposure to environmental pollutants has been potentially attributed to the worldwide prevalence and dramatic increase of obesity and type 2 diabetes over the last four decades. World Health Organization estimated 1.5 billion adults worldwide are overweight or obese and the number of type 2 diabetes increase from 153 to 347 million between 1980 and 2008. Earlier studies using animal models have reported that low doses of bisphenol A (BPA), phthalates, DDT, TBT and other persistent organic chemicals and some heavy metals such as cadmium, arsenic, lead etc., can make animals fat and gain weight. However, these chemicals cause similar harms to humans is still need to be elucidated. According to the 2015 Alliance for a Healthier Generation, the Commonwealth of Kentucky is the 7th most obese state with (35.7%) in the U.S. for children and 5th most obese state for adults (33.2%). Our study in western Kentucky dealing with the environmental levels of above mentioned chemicals were: 172-2950ng/g BPA in indoor dust; 6.8-356 ng total butyltins/g dry wt. sediment from Kentucky Lake; 32-107 ng total butyltins/g dry wt. in freshwater mussel tissues from Kentucky Lake; 1.65-1.81 ng BPA/L in Clarks River Water, Kentucky; 63-414ng total PCB/g dry wt fish from Kentucky Lake; 8.2-990 ng perfluorooctane sulfonate (PFOS)/g dry wt. solid waste samples from wastewater treatment plant from Murray, Kentucky; 11-130ng PFOS/mL blood sera of females and 19-164 ng PFOS/mL blood sera of males from Kentucky. The results show the presence of potential obesogens in environmental and biological tissues including human blood sera from western Kentucky, however, these levels cause obesity in human or animals is yet to be determined.

MP158 Framework for modeling the release of chemicals during the use phase of consumer products

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Chemicals contained in consumer products have become the focus of recent regulatory developments including the California Safer Consumer Products Act. Quantifying the amount of chemical releases during the use phase of consumer products, however, is a challenge, limiting our ability to understand the impacts of these chemicals used in consumer products. This study presents the current progress of the framework under development for estimating the release of chemicals during the use phase of consumer products when product-specific chemical release measurements are lacking. First, quantitative structure-function relationship (QSFuR) is developed using over 8,000 chemical-function pair data as training data. Second, the quantity of chemicals entering into different product streams is estimated based on market size data of nine major functional use categories. Third, chemical releases are estimated per each chemical use scenario using the Specific Environmental Release Categories (SPERCs) data developed by the European industry sector groups and trade associations. The framework currently considers 10 functional uses and linked them with 15 different products. Together with the complied release factors, the framework can provide quantitative estimated release amount to indoor air, outdoor air, wastewater, and soil for the input chemical. In the future, additional functional uses, market size data, and release factors will be further incorporated to improve the coverage and precision on release estimates for this framework. The framework helps understand the potential risk of organic chemicals in consumer products when empirical release factors are not available, and can help decision makers make informed decisions considering potential use phase impacts.

MP159 Hand-to-mouth contact frequency among adults: A review of the literature and recommendations for future studies

A. Gauthier, R.C. Lewis, R. Kalmes, Exponent, Inc.

Environmental agents may be transferred to the hands after contact with a contaminated surface, such as a consumer product. This transfer may consequently pose an opportunity for inadvertent ingestion should hand-to-mouth contact occur. A key determinant of exposure from this route is frequency of hand-to-mouth contact. In theory, the more frequent the hand-to-mouth contact, the greater the probability for exposure. Historically, due to their prevalent hand-to-mouth behavior, infants and young children have been the primary focus of research on this subject matter. Among adults however, this has been an under-researched aspect of human behavior in the peer-reviewed literature. The current study analyzed 11 key publications of adult hand-to-mouth behavior in adults. The analysis showed that the current state-of-the-science largely does not address the nuances of hand-to-mouth behavior that are essential for accurately characterizing exposures from this pathway. Although this information is necessary to accurately characterize exposures and resultant human health risks, and evaluating regulatory compliance in certain contexts (e.g., Proposition 65), many data gaps remain. Due to the lack of relevant data, investigators often rely on hand-to-mouth contact data that may not necessarily be contextually-specific, which can contribute to error in estimated exposures. Results from a recent hand-to-mouth study associated with specific consumer behaviors are presented. Additional more detailed research is needed on hand-to-mouth behavior in adults as this will greatly refine judgements of exposures and associated human health risks.

MP160 Health Risks from Chemicals During the Use Phase of Consumer Products – Current Knowledge and Information Gaps

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Health risk from chemicals in consumer products has become a major concern and gave rise to regulatory developments including the Safer Consumer Products Act. However, it remains a challenge for consumer, industry, and regulators to identify which chemicals should receive priority in scrutiny. This study reviewed previous literatures on chemical risks from the use phase of consumer products. Based on past research, this study aimed to elucidate what type of chemicals possess highest potential hazard for the use phase of consumer products. In addition, available research and regulation efforts on chemicals found in the review were summarize to identify potential chemicals that may be understudied. To match the scope of this study, the chemical should be 1) artificially manufactured; 2) added to consumer products to achieve certain functions; 3) not a drug or food; 4) route of exposure not originated from the general environment or occupational environment. 215 peer-reviewed papers in the past decade on chemicals commonly found in consumer products were reviewed and 122 unique individual chemicals or chemical groups were identified. The patterns between the chemical's functional use, product application, toxicity, and exposure routes are semi-quantitatively assessed and analyzed with Bayesian Inference. Randomly selected 122 chemicals from the USEPA CPCat database Chemicals were subjected to the same analysis to serve as control group. Results showed chemicals used in toys, baby products, personal care products, cosmetics, have evidence for carcinogenicity or causes cardiovascular disease, have multiple exposure pathways are more likely to be banned in the United States. Interestingly, rat oral LD50 value does not provide any indication of the likelihood for a certain chemical be banned in the United States. This study provided not only an overview of the most concerned chemicals, but also may help industries and regulators identify what combinations of product application, toxicity, and exposure of new chemicals would pose more risk to consumers.

MP161 Identification and Toxicological Evaluation of Unsubstituted PAHs and Novel PAH Derivatives in Pavement Sealcoat Products

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High concentrations of polycyclic aromatic hydrocarbons (PAHs) have been known to be contained in pavement sealcoat products. Unsubstituted PAHs are the most commonly detected PAH group in pavement sealcoat products. However, the analysis of PAH derivatives is still limited or none at all. This study is aimed at this research gap by determining the concentrations of PAH derivatives, including, ten heterocyclic-PAHs (Hetero-PAHs), twenty six nitrated-PAHs (NPAHs), and ten oxygenated-PAHs (OPAHs) in coal-tar and asphalt based sealcoat products, as well as time point scrapes after application of a coal-tar based product. Twenty three unsubstituted PAHs, ten methyl-PAHs (MPAHs), and eleven high molecular weight-PAHs (MW302-PAHs) were also analyzed. Benzo[a]pyrene-carcinogenic equivalent (B[a]P_{eq}) concentrations for the coal-tar based products was calculated and we found that, with the inclusion of MW302-PAHs not previously measured in sealcoat products, there was an increase of 4.1 to 38.7% in B[a]P_{eq} concentrations. We also hypothesized that photochemical transformation to NPAHs and OPAHs on sealcoated surfaces should be considered as degradation pathway of unsubstituted PAHs. The Ames assay was used to evaluate the mutagenicity of the samples. We found that the coal-tar based products were mutagenic, but not the asphalt based products. To evaluate the toxicity of sealcoat samples, the zebrafish developmental test was used. This toxicity test indicated that fractions where NPAHs and OPAHs eluted were associated with the most significant adverse effects. Overall, our results contribute to the assessment of the potential human health impact of pavement sealcoat products.

MP162 Identifying and addressing contaminant sources impacting an urban estuary

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Urban estuaries often harbor high concentrations of anthropogenic contaminants, resulting in elevated exposures and impacts to wildlife. Proactive, collaborative, and sustained monitoring of estuarine environments can aid in early detection of contaminants of concern, inform source identification and control options, and demonstrate the long-term impacts of control actions. The Regional Monitoring Program for Water Quality in San Francisco Bay (RMP) conducts special studies and status and trends monitoring in San Francisco Bay, California, USA, the largest estuary on the west coast of North America. Throughout its 25-year history, the RMP has maintained a strong collaboration among regulators, regulated dischargers including wastewater and stormwater agencies, and independent scientists, which has aided detection of contamination derived from consumer and industrial products before it causes major impacts to wildlife. The RMP develops and disseminates scientific information specifically relevant to source identification and potential control actions on contaminants such as flame retardants, perfluorinated stain-repellants and surfactants, pesticides, and personal care product ingredients. Long-term monitoring has also allowed the RMP to track the recovery of the Bay following management actions including chemical phase-outs and bans. This presentation will summarize common challenges to pollution prevention activities to highlight areas where research by independent scientists is needed. Examples include a) the lack of information on chemicals in consumer or industrial products; b) the diversity of uses and sources for many chemicals of concern; and c) early identification of chemicals of concern when data gaps for chemical production, environmental occurrence, and ecological impacts are common. As a respected source of independent science for regional and state water quality managers, the RMP serves as a clearinghouse for applied science that directly informs key management decisions to reduce environmental contamination.

MP163 Identifying Flame Retardant Chemicals in Polyurethane Foam From Consumer Products: Trends in Use Reflecting Shifts in the Flammability Standards

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Before 2005, PentaBDE was a common flame retardant (FR) additive in polyurethane foam containing residential furniture, and it's believed that the heavy use of PentaBDE in furniture in the US and Canada contributed to higher exposures among the countries populations. Following the phase-out of PentaBDE, the use of several alternate FRs increased, which included many organophosphate esters such as tris (1,3-dichloroisopropyl) phosphate (TDCIPP). However, in 2013 TDCIPP was banned from use in furniture in several US states. Around the same time, the State of California altered the residential flammability standard (TB 117) from an open flame test to a smolder ignition test. Given these changes, it is important to understand which chemicals (if any) are still being used in residential furniture in order to evaluate human exposure and health risks. In early 2014, the Duke University Superfund program developed a free public service to test polyurethane foam for FRs. This service has allowed us to build a database of FR use in consumer products and evaluate temporal changes in chemical use. As of May 2016, 1179 samples were analyzed, 50.9% of which contained an identifiable FR. Major product categories were sofas and love seats (456), chairs (120), mattress pads (117) and child car seats (99). TDCPP was the most frequently detected FR, followed by Firemaster®550 (FM550) > Tris (chloropropyl) phosphate (TCPP) > PentaBDE (a PBDE commercial mix). With the exception of 3 products, PentaBDE was not observed in products purchased post-2005, concordant with the 2005 voluntary phase-out. TDCIPP detection across all samples containing a FR dropped from 48.7% to 32.9% in products purchased before and after 2013, possibly reflecting industry response to California's 2013 change to flammability standard TB117 and/or the addition of TDCIPP to Prop65. Concomitant with the decrease in the use of TDCIPP we observed an increase in the use of TCPP as well as a non-halogenated aryl phosphate mixture containing isomers of tertbutyl phenyl diphenyl phosphate. We also report on the identification of a new FR, tetrakis(1-chloropropan-2-yl) (oxybis(ethane-2,1-diyl)) bis(phosphate), which we detected in 4.5% of FR-containing products, most of which were child products. These findings highlight trends in FR use and the value of this service to capture data on FRs in consumer products.

MP164 IFRA Environmental Standards and RIFM Program Advances Update for 2016

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To assure safety of fragrance ingredients in consumer products, International Fragrance Association expanded the fragrance industry's self-regulatory safety program with the development of IFRA Environmental Standards for both risk and hazard in 2008. Fragrance material risk assessments for these Standards are incorporated in the Research Institute for Fragrance Materials' (RIFM) testing program in coordination with its Expert Panel. To identify materials for risk assessment refinement, fragrance materialswere screened using the RIFM Environmental framework and 2008 IFRA volume of use survey as reported for both Europe and North America. The Framework for this evaluation was published in Environment Toxicology and Chemistry (Salvito et al., 2002, 1301-1308). In addition, hazard assessment on these materials was also performed and reviewed. As a result nearly 3,000 materials were screenedwith preliminary risk quotients estimated to rank priority materials for risk assessment refinement. In an effort to provide greater transparency to the IFRA Environmental Standards, RIFMreports the most recent results of these additional tests (for both risk and hazard assessments) at both the annual SETAC NA and Europe meetings. These studies include persistence testing (ready biodegradation tests and die-away studies), bioaccumulation, and acute and chronic aquatic toxicity. Incorporating these new data in a second tier risk and hazard assessment for these materialswill also be presented.

MP165 Integrating Exposure Science into Consumer Products Regulations

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California's Safer Consumer Products (SCP) program represents a new and transparent approach to regulating Chemicals of Concern in consumer products. SCP's regulations dictate that the California Department of Toxic Substances Control (DTSC) use a narrative standard to evaluate and identify product-chemical combinations on the basis of potential for exposure to a chemical in the product to contribute to adverse impacts to human health or the environment. This allows SCP to take a precautionary approach and integrate emerging data and approaches into the product-chemical prioritization process. In turn, SCP can influence the reduction of Chemicals of Concern in the environment by requiring manufacturers to consider the full life cycle of their products when selecting alternatives to avoid regrettable substitutions. There are an ever-increasing number of product-chemical combinations that are of concern for humans and the environment alike, but information about the source of these contaminants and relevant exposure pathways is often lacking. To be able to prioritize and list product-chemical combinations, SCP must have an understanding of human and environmental exposures and exposure pathways to Chemicals of Concern in consumer products. This talk will focus on how the SCP program uses exposure data to evaluate product- chemical combinations as well as the importance of exposure data for manufacturers completing an Alternatives Analysis. It will also highlight some of the reasons that this information is not always available, including analytical limitations, changing product formulations, and information gaps in conceptual models to understand the sources of environmental and human exposures.

MP166 Organophosphorus flame retardants: environmental occurrence, human exposure, and toxicity

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Polybrominated diphenyl ethers (PBDEs) have been gradually replaced with alternative flame retardants such as organophosphorous flame retardants (OPFRs) due to their persistence, bioaccumulation, and toxicities. OPFRs such as chlorinated and non-halogenated OPFRs, e.g. tris(2-chloroethyl) phosphate (TCEP), tris(1,3-dichloro-2-propyl) phosphate (TDCPP), tris(1-chloro-2-propyl) phosphate (TCPP), triphenyl phosphate (TPHP), resorcinol bis-diphenyl phosphate (RDP) and bisphenol A bis-diphenyl phosphate (BPA-BDPP), have been reported in various environmental media and in humans worldwide. In this study, information on physicochemical properties, occurrence in indoor environments, human exposure, and toxicity of these chemicals were reviewed, and areas warranting further investigation were identified. In house dust and indoor air, the highest levels of TCEP, TDCPP and TCPP were reported in Asia. Exposure sources for TPHP, RDP and BPA-BDPP in residence were electronic devices or furniture. In human urines, higher levels of diphenyl phosphate (DPHP) and bis(1,3-dichloro-2-propyl) phosphate (BDCPP) were detected in children than in adults. Urinary DPHP and TPHP levels were associated with those detected in floor dust or hand wipes. In vitro and in vivo studies showed endocrine disrupting potential of TPP, TCEP, and TDCPP. Limited epidemiological evidences suggest adverse effects on reproduction. However, for TCPP, RDP and BPA-BDPP little is known about their toxicities. Considering increasing levels of exposure to these chemicals among humans, efforts are warranted to identify their exposure sources, and to investigate toxicological and health consequences.

MP167 Sources of Fipronil in Urban Aquatic Environments

K. Moran, TDC Environmental, LLC

Currently used pesticides have been found in urban runoff, municipal wastewater, and urban aquatic environments throughout the USA, sometimes at concentrations that exceed aquatic life protection benchmarks. Fipronil has among the highest detection rates for insecticides in California urban runoff and urban waterways. Recent measurements have revealed the presence of fipronil in California municipal wastewater influent, effluent, and sludge. In urban runoff, municipal wastewater effluent, and urban waterways total fipronil concentrations are reaching—and in some cases exceeding—levels known to cause toxicity to sensitive aquatic organisms. In California, fipronil has relatively few approved uses—it is only registered for structural pest control and pet treatments; it is not approved for agricultural or general landscape use. Its short list of allowable uses simplifies the process of identifying surface water exposure pathways. A preliminary conceptual model of fipronil use and transport from urban areas to surface waters was developed, based on a literature review, pesticide product labels, environmental fate data, California pesticide sales and reported use data, pesticide retail shelf surveys, and wastewater and urban runoff monitoring data. Fipronil can be washed into indoor (sewer) and outdoor (storm) drains during or after outdoor pesticide applications or as a consequence of pesticide handling activities. Source investigations guided by the model clarified the relative magnitude of each fipronil source. Growing evidence suggests that the primary source of fipronil in urban runoff is treatment of impervious surfaces around buildings–particularly surfaces that drain across paved areas to piped storm drain systems (i.e., directly connected impervious areas like building driveways and front walkways). Wastewater influent monitoring data, product information, and data from the literature characterizing transfer from application locations to other surfaces suggest pet flea and tick products are the primary source of fipronil in municipal wastewater. The refined conceptual model, which now reflects major fipronil sources and aquatic exposure pathways, provides a framework to guide development of strategies for reducing urban fipronil water pollution. The growing scientific understanding of fipronil pathways to surface water advances insights into exposure pathways for other products used in similar locations in urban environments.

MP168 Sunscreen Safety for Kids: A Risk Assessment of a Common Preservative

J. Lemay, K. Zu, Gradient; L. Zhang, VU Univ Amsterdam / Animal Ecology; J. Goodman, Gradient

Phenoxyethanol is a common preservative used in cosmetic products to prevent bacterial growth. Research and regulatory agencies worldwide conclude that phenoxyethanol poses no risk if it makes up less than 1% of a cosmetic product. Most recently, the European Union affirmed the safety of phenoxyethanol for use as a preservative with a maximum concentration of 1%. Despite these assertions, concern has arisen that the regulatory limit for phenoxyethanol may not provide an adequate margin of safety for infants and small children. Therefore, we conducted a hazard and risk assessment for phenoxyethanol in children's personal care products. We first conducted a hazard assessment of phenoxyethanol in its neat form, considering both dermal and oral exposure. We found that, although no studies have directly evaluated the carcinogenicity of phenoxyethanol, available evidence indicates a lack of genotoxicity. Also, laboratory animals dermally exposed generally experienced no adverse effects. Following oral exposure, some toxicity occurred at very high doses. In two-generation studies, no adverse effects were observed in offspring unless the dose was high enough to cause maternal toxicity. We identified the points of departure (PODs) of phenoxyethanol from the hazard assessment, then calculated children's exposures from using multiple personal care products containing phenoxyethanol. We evaluated one common brand and divided its products for children into two broad categories: lotions/creams and bathing products. For each category, we selected the products with the highest concentrations of phenoxyethanol to calculate hypothetical exposures. We estimated typical exposures possible for a child using regular lotions, sunscreen lotions during time outdoors, and body wash at bath time. Our margin of exposure (MOE) analysis determined that the typical exposure scenarios were more than 200 times less than to the PODs identified in the hazard assessment. We conclude that the available evidence indicates that phenoxyethanol, present at < 1% of formula in personal care products, does not pose a health risk to infants or small children.

Business Examples of Chemical Alternatives Assessment

MP169 The Sustainability Consortium – Common Chemical Evaluation Framework

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Consumer and advocacy group concerns related to the potential human health and environmental impacts associated with chemical exposure are growing in the home and personal care industry. These concerns are reflected both in retailers' sustainability policies and commitments around chemicals and in manufacturers' attentiveness to the development of "greener" products. Accordingly, retailers and brand manufacturers have focused on identifying high priority chemicals, disclosing these chemicals to consumers, and promoting safer ingredient choices through alternatives assessment and informed substitution. Though these efforts aim to provide progressive solutions to specific concerns regarding chemical hazards, unilateral action by individual retailers or manufacturers increases the risk of gridlock, system inefficiency, and lack of market acceptance of any given solution. To ensure that actionable and lasting progress can be made, The Sustainability Consortium (TSC) has convened members and invited participants to develop a framework that uses best and transparent approaches for ingredient selection and product safety evaluation. The framework, presented in this paper, consists of four facets of ingredient and product assessment – function, list inclusion, hazard assessment, and risk assessment – with a process flow chart that provides a means for enhanced transparency and support for evaluating and choosing ingredients to create safe products that function as intended. When completed, the framework could be used by retailers, manufacturers, and NGOs in a multitude of ways: to facilitate decision making for ingredient and product safety evaluation, as a decision support tool for product/ingredient development to create safe products, as a basis for defining leadership levels and activities, and as a communication tool to disclose current approaches for ingredient selection and product safety evaluation. Ultimately, when implemented in combination with current TSC metrics, this framework will contribute to enhanced communication on chemical safety issues throughout the value chain while driving the development of safe and more sustainable products.

MP170 Examination of three approaches for selecting candidate chemicals for an AA

A.M. Mason, American Chemistry Council / CPTD; B. Howard, American Chemistry Council / Value Chain; S. Arnold, The Dow Chemical Company; S. Risotto, American Chemistry Council; T. Kingsbury, TKingsbury LLC

As the community of practice identifies and assesses candidate chemicals for new product development, it faces a variety of challenges when evaluating alternatives. On the one hand there is a desire to reduce and/or eliminate hazardous ingredients and create new products applying practices in green engineering and chemistry; on the other hand there is a need to meet customer expectations and to develop a product that performs effectively and is cost efficient. Overall, the desired outcome for any assessment of alternative chemicals or products is to identify those that perform similarly or better than its predecessor, be measurably safer for the user and the environment, and have an economic profile comparable to the chemical or product it replaces. Assessors can choose a set of candidate chemicals using a number of approaches: 1) use hazard alone, 2) use a comparative combination of hazard and exposure, or 3) use a more expanded set of criteria that include hazard and exposure plus performance and product use. This presentation will use two generic product formulations to outline the information from each of these three approaches to demonstrate how the decisions change with the approach used.

MP171 Automated human and environmental exposure estimation to support prioritization of chemical alternatives in product development

J.P. Rinkevich, P. Beattie, J. Orchard-Hays, M.C. Ruhter, SciVera LLC

New regulations and market pressures are driving companies to implement enhanced business processes that improve understanding of the human and environmental health characteristics of chemicals in products and processes. Chemicals considered for use in products and processes present varying hazard characteristics for human and/or environmental endpoints. Some chemicals may show high hazard for one endpoint while others may indicate high hazard for other endpoints. These varying characteristics offer opportunity for exploration of hazard-related tradeoffs between alternative chemicals in specific product or process applications. In addition to a review of chemical hazards, other attributes such as technical and functional performance, market availability, and cost factor into the viability of alternatives. The practice of Alternatives Assessment (AA) can incorporate contextual review of chemical use and predicted exposure to facilitate prioritization of alternative chemicals for a specific application. Doing this work at scale, for dozens or hundreds of chemicals, requires automation for reasons of efficiency and economy. Screening-level exposure estimation and corresponding risk characterization, when automated via computer software, can assist in the rapid, consistent, and cost-effective processing of AAs where human and environmental hazard characteristics vary across potential alternatives in a common application. This talk will present specific examples using the cloud-based software SciVeraLENS® to illustrate automated exposure estimation. The examples presented will show how automated screening-level exposure assessment and risk characterization can support the growing need throughout the consumer product value chain to evaluate chemicals in context of use, at scale, to enhance product and process sustainability attributes.

MP172 Case Studies in Reducing Aquatic Risks of Personal Care Products Using the GAIA Algorithm to Drive Selection of Alternative Ingredients

J.K. Saxe, EcoSafety Sciences; R. Predale, L. Ricicki, Johnson & Johnson Consumer Companies; R. Sharples, Johnson & Johnson Medical Devices; D.J. Caldwell, Johnson & Johnson / Environment Health Safety Sustainability

Personal care products (PCPs) are used globally and are typically emitted to the environment in wastewater under normal use. As water quality concerns due to industrial point sources decrease, regulators and scientists have begun focusing on this class of compounds as a possible water quality concern because of its widespread use. Businesses selling PCPs thus have a motivation to compile, interpret, and use any available data on potential risks to the natural environment as a guide for sustainable decision making regarding ingredients to select or avoid in PCPs, to minimize the potential for adverse environmental effects. We describe how the Global Aquatic Ingredient Assessment (GAIA) algorithm for scoring environmental hazards is used as an initial decision tool, and then, resulting potential risk due to the selected PCP ingredients is checked. GAIA incorporates information on environmental persistence, bioaccumulation potential, aquatic toxicity of the parent compound and degradants, excess toxicity from ecological endocrine disruption effects, and the potential for producing photochemical smog. The algorithm yields numeric scores that discern modest differences in environmental hazard potential by aggregating empirical and modeled data for disparate endpoints using a system of weighting and penalties. Because of its ease of use by non-experts in environmental risk assessment, GAIA has been used as a front-line decision tool by formulators; however initial hazard-based decisions are prone to certain errors, which have been avoided through downstream risk assessment using several different approaches, depending on business needs. Case studies on the use of GAIA plus additional data to guide product development include: (1) the combination of GAIA scores with product efficacy yields an initial decision framework with an estimate of relative risk, demonstrated for two bath cleansing products; (2) competing shampoo formulations, driven by hazard-based GAIA scores, are compared in terms of relative aquatic risks, which are estimated using effluent modeling and risk ratios, demonstrating significant risk reductions; and (3) the absolute incremental risk due to the use of surfactants in a shampoo is estimated for commodity surfactants having less favorable GAIA scores and their alternative, a novel surfactant having a more favorable GAIA score, by using effluent modeling, environmental monitoring data, and risk ratios, confirming benefits of the alternative.

MP173 Alternatives Assessment of non-fluorinated DWR Products utilizing the GHS-Column Model

R.C. Buck, The Chemours Company / Fluoroproducts; K. Schubert, The Chemours Company

Textile brands and retailers are facing increasing pressure from stakeholders to replace a number of chemicals in their supply chain with “safer alternatives”. Many brands and retailers face a dilemma because they often have no “easy to use” tool in their hands to assess whether or not an alternative might be a preferred choice. The GHS-Column Model is a no cost practical tool that could fulfill this need. This hazard-based model was developed by the Institute for Occupational Safety and Health of the German Social Accident Insurance (IFA) and provides clear and practical guidance on how to rank six different hazard factors on five different levels, including ranking for data gaps. The GHS-Column Model is a quick and facile first level assessment to screen in or screen out potential alternatives based on hazard data from a product safety data sheet (SDS). The GHS-Column Model was utilized to assess 11 different non-fluorinated durable water repellent (DWR) products. Results of this assessment will be presented and context will be provided how the outcome can benefit downstream customers to identify potential substitutes on their journey to better alternatives.

Scientific Advances Supporting Aquatic Life Water Quality Criteria Derivation

MP174 Advancing Uncertainty Characterization in Support of Aquatic Life Water Quality Criteria Derivation: Why and How

D. McLaughlin, NCASI

Numeric water quality criteria (WQC) derived to protect aquatic life represent potentially useful tools for the management of water quality and aquatic ecosystems. Ideally, these criteria reflect a sound scientific rationale using the best available science and provide useful estimates of pollutant levels that present unacceptaable risks to these ecosystems. An important challenge for the criteria derivation process is the characterization and communication of uncertainties in the science that supports numeric criteria. Without meaningful information on uncertainties, the utility of a numeric criterion for water quality management cannot be sufficiently assessed. This is especially important for criteria for substances that are derived using limited empirical data. Existing procedures used by the U.S. Environmental Protection Agency to derive numeric WQC such as the 1985 Guidelines (Stephan et al. 1985) provide little guidance on quantitative methods that may be used estimate uncertainties. Expanding this guidance would improve the transparency of aquatic life criteria, provide a more complete assessment of the state of the underlying science, and could help prioritize areas of research needed to improve the predictive value of aquatic life WQC. This presentation will review the limitations of uncertainty characterization in some published USEPA aquatic life WQC documents, and present examples illustrating the use of Monte Carlo simulation, binary classification techniques, and receiver operating characteristic curves that may be used to address these limitations.

MP175 Applying Adverse Outcome Pathways (AOPs) to Derivation aquatic PNECs for Two Typical Phenolic EDCs

Y. Wang, J. Wang, National Marine Environmental Monitoring Center/ China

More recently, adverse outcome pathways (AOPs) has been proposed as a novel concept that effectively take the consideration of toxic mode of action (MoA) and aid to guide ecological risk assessment (ERA) of chemicals. Assessing ecological risk of endocrine disrupting compounds (EDCs) is increasingly important due to its ubiquity in aquatic environment and endocrine effects on organisms. In the present study, from the point view of AOP, we derived aquatic predicted no-effect-concentrations (PNECs) of two typical phenolic EDCs, i.e. bisphenol A (BPA) and 4-nonylphenol (NP). Laboratory chronic toxicity data for BPA and NP on aquatic organisms were collected and grouped by whether they are associated with AOP, based on which endpoints include not only reproductive effects but also biochemical, genetic effects. By log-normal statistical extrapolation method, we calculated aquatic PNECs of BPA with values of 0.6 and 7.04 ?g/L, and NP with values of 0.22 and 3.9 ?g/L for AOP-related and non-AOP-related endpoints, respectively. The present results showed that classification of toxicity data can provide lower AOP-related PNECs and keep aquatic organisms from adverse effect such as endocrine disrupting effect more appropriately. The ecological risk assessment framework raised in this study can also be applicable for other types of EDCs with different molecular initiating events such as binding with androgen receptor and thyroid hormone receptor.

MP176 Aquatic fungal secondary metabolites contamination of water

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Fungi are ubiquitous microorganisms and they can be found in food, environment, water etc., and they are classified as heterotrophic organisms since they cannot make their own food. Some waterborne fungi produce antibiotics or compounds that can be used as soil improver but some produce toxic secondary metabolites (mycotoxins) and may therefore pose a hazard to both animals and humans. The presence of harmful fungi in drinking water may result in changes in water taste and odour among other negative effects. In this work, water samples were collected from Roodeplaat Dam in Gauteng province and they were transferred on potato dextrose agar (PDA) growth media supplemented with streptomycin sulphate for inhibition of bacterial growth. DNA was extracted directly from fungal mycelium followed by polymerase chain reaction and detected using the primer pair ITS1F (forward) and ITS4 (reverse). The smears obtained from 1% agarose gel showed the presence of fungal DNA in samples collected from Roodeplaat Dam. Both beneficial and toxic fungal species were found to be present in the sample as identified after Sanger sequencing. Two bands were obtained from the gel and they corresponded to Talaromyces flavus and Penicillium ochrochloron. In addition, extracellular metabolites produced by these fungal species were extracted using solid phase extraction (SPE) method from Dam water and identified using high resolution liquid chromatography quadrupole time of flight mass spectrometry (LC-QTOF-MS) and NMR for further confirmation of the metabolites.

MP177 Derivation of BLM-based Ambient Water Quality Criteria for Lead Following USEPA Guidelines: A Comparison with European Approaches

D.K. DeForest, Windward Environmental LLC; K. Brix, EcoTox / Zoology; P. Van Sprang, ARCHE; R.C. Santore, Windward Environmental, LLC; M.J. Chowdhury, International Lead Association / Manager Environment

The U.S. Environmental Protection Agency's (USEPA's) current ambient water quality criteria (AWQC) for lead (Pb) in freshwater were developed in 1984. The criteria are adjusted for hardness, but more recent studies have demonstrated that other parameters, especially dissolved organic carbon (DOC) and pH, have a much stronger influence on Pb bioavailability. These recent studies have been used to support development of a biotic ligand model (BLM) for Pb in freshwater, such that acute and chronic Pb toxicity can be predicted over a wide range of water chemistry conditions. Following USEPA guidelines for AWQC development, and using a methodology consistent with that used by the USEPA in developing its recommended BLM-based criteria for copper in 2007, we propose acute and chronic BLM-based AWQC for Pb in freshwater. In addition to the application of the BLM approach that shows ability to better account for site-specific Pb bioavailability, the toxicity datasets presented here are also much more robust than in 1984 and there are now sufficient chronic Pb toxicity data available that use of an acute-chronic ratio (ACR) is no longer necessary. Over a range of North American surface waters with representative water chemistry conditions, proposed acute BLM-based Pb criteria ranged from approximately 20 to 1000 µg/L and chronic BLM-based Pb criteria ranged from approximately 0.3 to 40 µg/L. Differences exist between US and European approaches used for driving AWQC or aquatic safe thresholds of metals. The key differences include the use of genus means, EC20s, and exclusion of algae and plants from the sensitivity distribution in the US versus the use of species means, NOEC/EC10s, and inclusion of algae and plants in the sensitivity distribution in the EU. Additionally, the approach to distribution fitting is also different. In order to understand the implication of methodological differences, a set of ecological threshold concentrations of Pb derived for selected European freshwater scenarios will be compared with the proposed chronic Pb AWQC.

MP178 Development of Site-Specific Water Quality Criteria: A Detailed Look at Water-Effect Ratio (WER) Testing and Application

J. Nusz, Exponent, Inc.

Water quality standards (WQSs) more generally applied (i.e. national, tribal or state-mandated) for the protection of aquatic biota may be overprotective or underprotective, depending on the specific conditions present at a given site. Site-specific water quality criteria (SSWQC) may be more appropriate when, for example, species native to the site are very different than species used to derive the more general WQSs, naturally occurring pollutant concentrations in a stream segment exceed the national criteria for aquatic life uses, or when site-specific water characteristics may affect the toxicity of contaminants. USEPA Regulation 40 CFR 131 allows states and tribes to develop site-specific water quality criteria (SSWQC) and generally grants approval if the criteria are supportive of the designated water uses and are based on supported scientific rationale. This is often reflected in a permit (e.g. NPDES) or developed for specific bodies of water. Additionally, SSWQCs can be developed for approval by local governments as a part of the Environmental Impact Assessment (EIA) process necessary for the international permitting of many types of facilities. There are several scientifically justified approaches for development of SSWQC development for which EPA provides guidance (e.g. recalculation procedure, water-effect ratio (WER) procedure and resident species procedure). Deciding which approach to take depends on the characteristics of the site in question as well as the types of data available. Each of these approaches and circumstances under which they may be efficiently applied will be presented. Furthermore, a hypothetical example application of the WER procedure for deriving a SSWQC for a tailings pond discharge permit, under international context, will be provided.

MP179 Development of Water Quality Guidelines for Copper Using the Biotic Ligand Model

S. Dixit, Environment and Climate Change Canada / Environment and Climate Change Canada; R.C. Santore, Windward Environmental, LLC

The bioavailability and aquatic toxicity of metals can be influenced by physical and chemical characteristics of the water such as hardness, pH and dissolved organic carbon. The National Guidelines and Standards Office (NGSO) of Environment and Climate Change Canada has developed a number of water quality guidelines (WQGs) for the protection of aquatic life for metals and is evaluating the latest tools and approaches for considering toxicity modifying factors (TMFs) in guidelines derivation. Single-variable regression analyses were considered in the past to account for individual TMF (e.g., hardness), followed by the incorporation of multiple TMFs (e.g., hardness, pH, DOC) using multiple linear regression (MLR). The NGSO is now considering the biotic ligand model (BLM) approach for developing long-term water quality guidelines for copper. The BLM allows consideration of a diverse set of water quality parameters which are known to impact copper toxicity, including pH, DOC, calcium, magnesium, sodium, carbonates, etc. The approach taken to develop a BLM-based water quality guideline for copper involved compilation and evaluation of chronic toxicity data following the CCME guidance (2007), evaluation of model performance with the chronic toxicity dataset, and utilization of the BLM to normalize the chronic toxicity dataset to site-specific chemistry conditions to construct species sensitivity distributions (SSD).

MP180 Frameworks for Addressing Aquatic Life Effects from Time-Variable Toxicant Exposures

C.G. Delos, Great Lakes Environmental Center / Mail Code

USEPA's existing procedures for deriving aquatic life criteria, last revised in 1985, offer little rigor for addressing the time variability of exposure, and lack reproducible procedures for deriving either the averaging period or the target frequency of attainment, two of the three components of a criterion. In the context of aquatic life criteria development, this work describes approaches that can offer a unified perspective on the affected percentage of species, individuals, and time. It discusses differences between simple approaches relying only on a statistical distribution of concentrations, and complex approaches that, in time-variable applications, can (a) normalize the effects on life stages having different sensitivity and duration, (b) normalize for chronic lethal versus sublethal effects, and (c) account for innate differences among species abilities to replace individuals lost to toxicity (that is, differences in recovery time). This work describes practical approaches that can be applied to national, state, and site-specific criteria.

MP181 How do organisms regulate accumulation of toxics, pharmaceuticals, and nutrients?

W. Beckon, US Fish and Wildlife Services / Division of Environmental Contaminants

All kinds of organisms exert a simple form of non-homeostatic regulation over their internal concentrations of a wide variety of substances: essential elements, toxic metals and other contaminants, and even novel pharmaceuticals. At higher exposure concentrations, organisms incorporate proportionately less of the chemical to which they are exposed. This regulation occurs regardless of whether the exposure is dietary or more direct environmental exposure. Thus, in general, bioconcentration "factors", bioaccumulation "factors", and trophic transfer "factors" are not constants, but steadily decline with increasing exposure. Evidence collected so far suggests that this phenomenon is usually due to a decline in the uptake rate "constant" and/or, usually to a lesser extent, an increase in the depuration rate "constant" with increasing exposure. Therefore, contrary to common expectations, first order kinetics are not generally adequate to describe the processes of uptake and depuration. The physiological bases for this widespread form of regulation remain to be clarified.

MP182 Refinement and Validation of Target Lipid Model-Derived HC5 to Conform to REACH Guidelines

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The Target Lipid Model (TLM) has been extensively validated for predicting the toxicity of narcotic chemicals, such as petroleum hydrocarbons, and the USEPA has adopted the TLM framework for deriving water and sediment guidelines for chemicals that demonstrate a narcotic mode of action. The TLM was extended to derive HC5 values, concentrations below which only 5 percent of species should be adversely affected. The European Chemical Agency identified several shortcomings where the derivation of the TLM-derived HC5 did not conform to the requirements under the REACH regulation. These shortcomings included insufficient species representation in the acute and chronic toxicity databases, the use of chronic endpoints that were not appropriate for use in risk assessment, databases of critical target lipid body burdens (CTLBB) and acute to chronic ratios (ACRs) were not log normally distributed and the omission of a co-variance term in the HC5 equation. These shortcomings were addressed by expanding the acute and chronic databases to include the required taxonomic groups and increase the overall number of observations and revising the HC5 equation as appropriate. The resulting CTLBB and ACR databases conformed to the requirements under REACH for use in risk assessment. The revised HC5 values are scientifically valid and were demonstrated to be protective of chronic effects from exposure to petroleum hydrocarbons.

MP183 The Effects of Sensitivity Bias in Species Sensitivity Distributions and Possible Effects on Water Quality Guidelines

K.E. Croteau, D.K. DeForest, R.C. Santore, Windward Environmental, LLC

Since the mid-1970s, thousands of studies have evaluated the toxicity of various chemicals to aquatic organisms. Results from many of these studies have been used to develop species sensitivity distributions (SSDs) for deriving water quality guidelines. Recently, there has been more emphasis on evaluating the toxicity of sensitive organisms rather than the entire range of sensitivities. The SSD is intended to inform the derivation of criteria for the protection of all species, not just those that were included in the SSD. The over-emphasis of the more sensitive end of the SSD can contribute to a skew in the observed distribution such that the shape of the distribution is distorted from what it would be if all species could be tested, which ultimately affects the derived criterion value. The way in which the SSD is analyzed could potentially mitigate the effects of the sensitivity bias, but to what extent? Although most parties use a specific percentile of the SSD (e.g., 5^{th} percentile) as a regulatory benchmark, there is not a global consensus on what method should be used to derive that benchmark. The type of statistical distribution that is assumed and how much of the dataset (i.e., full or truncated) is used for defining the 5^{th} percentile of the distribution are two ways in which the United States and European Union differ in their approaches. How much do these choices of distribution and truncation impact the 5^{th} percentile, with and without an over-emphasis on the sensitive end of the SSD? This presentation will attempt to answer these questions through numerical examples and statistical resampling of data. We will discuss how an emphasis on more- or less- sensitive organisms in the SSD would affect the 5^{th} percentile estimate's conservatism, and how the method of deriving the 5^{th} percentile influences the outcome.

MP184 Use of SeqAPASS for the Identification of Susceptible Taxa to Inform Derivation of Aquatic Life Criteria

C. LaLone, USEPA / Mid Continent Ecology Division; D.L. Villeneuve, G.T. Ankley, USEPA / National Health and Environmental Effects Research Laboratory

The 1985 U.S. Environmental Protection Agency (EPA) Guidelines for Deriving Aquatic Life Criteria (ALC) require acute and chronic toxicity testing with a fixed list of taxa that cover aquatic organisms from vertebrates, invertebrates, and plants. In considering Guideline revisions, there is interest in employing new and readily available technologies to inform chemical-specific selection of test species based on scientific evidence for susceptibility as a means to eliminate unnecessary animal testing, reduce cost, and gain the most applicable data for decision making. Further, when toxicity testing is completed on selected organisms, advances in methods for species extrapolation could be implemented to gain a greater understanding of how representative the data are of other species from the same taxonomic group. A publically available computational application for informing such considerations is the U.S EPA's Sequence Alignment to Predict Across Species Susceptibility (SeqAPASS; https://seqapass.epa.gov/seqapass/) tool. SeqAPASS facilitates cross species comparisons of chemical molecular targets based on evaluation of protein similarity. The underlying assumption is that if a chemical is known to act on a specific protein to initiate toxicity in one species, conservation of that protein in another species provides a line of evidence that the second species could also be susceptible to a given chemical. Depending on the degree of characterization of the selected protein and existing knowledge about the chemical-protein interaction, SeqAPASS derives susceptibility predictions by evaluating similarity from alignments of primary amino acid sequences, functional domains (e.g., ligand-binding domain), and individual amino acid residue(s) important for maintaining protein structure or for direct interaction with the chemical. Such data can be used to identify species that are likely (or unlikely) susceptible to a given chemical, therefore aiding in selection of appropriate test species. Additionally the data would be useful in understanding how broadly toxicity test data derived from one species may be extrapolated to others. In short, evaluation of cross species protein and structural conservation using SeqAPASS provides a rapid and cost effective method for deriving a line of evidence for chemical-specific testing strategies that may be useful in the revised ALC Guidelines. The contents of this abstract neither constitute nor reflect official USEPA policy.

MP185 Variability and Uncertainty in Use of Mean Effect Concentrations for Aquatic Life Criteria Derivation

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The acquisition of acceptable toxicity test data from the open literature to fulfill taxonomic diversity requirements representing a minimum eight families (the MDR) is a critical element in aquatic life criteria (ALC) derivation. In EPA's criteria derivation procedure, the geometric mean of all acceptable test level data for a species is the Species Mean Value (SMV), and the geometric mean of all acceptable species level values is the Genus Mean Value (GMV). The genus level values are arranged in a sensitivity distribution (SD), and the final acute or chronic value (FAV, FCV) is the 5th centile concentration of that SD. To date there has been no formal analysis of the reliability of mean toxicity values for representing sensitivity of species at any taxonomic level. It is not uncommon for a single acute or chronic effect concentration from a single toxicity test with a species to serve as the basis for the SMVs or GMVs. To better understand the variability and uncertainties in the use of mean toxicity values for ALC development, we examined several chemical-specific acute criterion datasets to determine the variability in the underlying values used to calculate Species Mean Acute Values (SMAVs) and Genus Mean Acute Values (GMAVs). The analysis focused on variability of LC50s, variability of SMAVs within a genus, and variability of GMAVs within a family. Our recommendation based on preliminary findings is that the variability of LC50s within a species suggests that an SMAV based on a single LC50 is too uncertain to use for criteria derivation, and that it is advisable to use a surrogate species whose SMAV is based on three or more LC50s, provided an SMAV for more than one species within the genus is available. The applicability and ramifications of our recommendation to re-calculation of the acute criterion will be presented, as well as findings from a similar exercise to better understand the variability and uncertainties in the use of mean toxicity values for chronic criterion development.

Environmental Chemistry

MP186 Supercritical CO2 Extraction and GC-MS Analysis of Fatty Acids Composition of the Oil from Leaves, Seeds and Husks of Moringa oleifera

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There has been a significant role played by plants in maintaining human health, and improving the overall quality of human lives for decades. Humans have used plants as traditional remedies to alleviate illnesses and promote the overall quality of lives due to the belief that plants have natural substances in them capable of promoting human health. Moringa oleifera has been shown through prior research to be a medicinal plant with a difference. It is a multipurpose tree used as spice, for cooking, as cosmetics oil and more importantly as medicinal plant. Moringa oleifera plant cultivated in Nigeria were characterized for the presence of biologically active phytochemicals and fatty acid contents using supercritical CO_2 extraction technique to extract the plant parts and GC/MS for structure elucidation of the compounds found. The extracts of the plant were characterized for the presence of fatty acids like oleic acid, linoleic acid, linolenic acid, hexadecanoic, hydrocarbons etc. The crude oils were derivatized by transesterification process to convert them to their corresponding fatty acid methyl esters (FAMEs) by methylation method using 1% boron trifluoride in methanol. FAMEs were analyzed using gas chromatography equipped with mass spectrometry (GC-MS) and eleven fatty acids were found in all samples. The dominant saturated fatty acid found in all samples was palmitic acid ranging from 7.8% of the total fatty acids in seeds to 22.4% in leaves. The leaves contain the chemical group of most interest; Linolenic acid (18:3n-3) which is the omega-3 polyunsaturated fatty acids at 37%. The major monounsaturated fatty acid in the seeds and husks were Oleic acid (18:1n-9), an omega 9 fatty acid at 79% and 77% respectively. All samples contained the following saturated fatty acids; palmitic (C16:0), palmitoleic (C16:1n-9), stearic (C18:0), arachidic (C20:0), and behenic (C22:0) acids. Several other fatty acids were detected in all samples at trace amounts. The presence of these bioactive and therapeutically potent compounds make Moringa oleifera a therapeutically potent plant in which further study of the plant may lead to development of novel agents used to treat various disorders.

MP187 Modulation of the physiological and biochemical effects of copper nanoparticles in kidney beans (Phaseolus vulgaris) treated with kinetin

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The environmental fate and effects of nanoparticles (NPs) are not well understood, despite their increased usage throughout modern technology. Long term studies on plants exposed to NPs under realistic environmental conditions is lacking. Within agriculture, copper (Cu) NPs have been indicated as a potential solution towards fungal and bacterial remediation. Plant hormones are also sought as a bio-based alternative to improve crop quality and yield. Kidney beans (Phaseolus vulgaris) are an important food crop that is widely produced and consumed worldwide. In this study, kidney bean plants were grown in potting soil treated with copper nanoparticles (nCu), bulk copper (bCu) and copper chloride ($CuCl_2$) at concentrations of 50 and 100 mg/kg^{-1} for 45 and 90 days. At 15 days of growth, 10 and 100 µm of kinetin (KN) was applied to plants. Plant tissue samples were harvested during the juvenile stage, and seeds were collected at the time of maturity. Physiological and biochemical parameters were investigated. Enzyme activities and chlorophyll content were evaluated through ultraviolet-visible spectroscopy (UV/vis) as plant stress indicators. Cu uptake and translocation were determined via inductively couple plasma-optical emission spectroscopy (ICP-OES). Seed micro and macronutrients were also quantified. There was a decrease in selenium for the 10 µm KN+100 ppm $CuCl_2$ treatment and all 100 µm KN treatments, under detectable limits with current methodology, as well as a 19 to 22 percent reduction in zinc for nCu treatments at both KN concentrations. Protein content was found to be higher in bCu treatments (27.92 and 28.23%) and lowest in the 100 µm KN+100 ppm $CuCl_2$ treatment (20.35%). For future work, KN content in soil and plant tissues will be studied. While results demonstrate thus far that the combination of nCu and KN is not significantly toxic to kidney bean plants, bCu and $CuCl_2$treatments have shown negative impacts.

MP188 Accumulation of metals in the lichen Flavoparmelia caperata

D. Brown, Marietta College / Biology and Environmental Science; J. Ste Marie, Marietta College

This study assessed the accumulation rate of metals in transplanted lichens (Flavoparmelia caperata) in relation to distance and direction from a ferro-manganese alloy factory in the Ohio River Valley. F. caperata collected at a control location was rinsed and placed in plastic mesh packets and exposed at various locations. Exposures lasted from one to 6 months, and it was found that by 5 months the transplanted lichens were reaching steady state, with regards to metal concentrations. By 5 months the metal levels in transplanted lichens were also approximately the same as lichens obtained from the transplant locations. Distance from the manganese source was strongly correlated with accumulation, with the closest transplant site having the highest concentrations of Mn (288.18 µg/g). Lichen samples were acid digested, and metal concentrations (Cr, Cu, Fe, Mn, and Ni) were determined using atomic absorption spectroscopy. Exposures were also conducted each month for a complete calendar year to look at seasonal variations in accumulation. The purpose of this part of the study was to compare accumulation of metals in lichens transplanted one kilometer to the north, south, east and west of the point source and relate those levels to wind direction and precipitation during the exposure period. Weather data for the area was obtained from a NOAA weather station located just outside of the Ohio River Valley. Based on prevailing winds from the southwest, we expected the highest metal concentrations to be observed to the north and east of the ferro-manganese alloy factory. However, for more than half of the months the highest metal levels were found at the western transplant site. We are currently working to understand how local weather conditions in the river valley may have impacted transport and deposition, to better understand why the observed results did not match what was expected.

MP189 The Effect of Cations on the Extractability of Organic Chemicals Adsorbed to Soil

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Sorption is an important factor in determining the potential impact of chemicals in the environment. There are many factors that affect the adsorption of chemicals to soil including, but not limited to; size, shape, molecular structure, polarity, solubility, configuration, polarizability and charge distribution of a chemical. Furthermore, soil has many characteristics that affect adsorption including cation exchange capacity (CEC), pH, clay content, particle size, ionic strength, as well as others.

Although hydrophobic partitioning has been recognized to have a large role in sorption mechanisms, other processes such as cation exchange and surface complexation with cations also have a role in sorption to soil. Exchangeable cations commonly found in the environment (Na^+, Ca^{2+}, Mg^{2+}, K^+) influence the adsorption of organic chemicals to soil. The current study utilizes different cations found in the environment to extract organic chemicals that are adsorbed to soil for analysis. This technique uses the cation exchange capabilities of the soil to remove the organic molecule by substituting the cation on the soil for the cation that is in the extraction solvent. Different cations were tested with various oxidation states including K^+, Na^+, NH_4^+, Mg^{2+}, Fe^{2+}, and Al^{3+}. Seven organic chemicals were tested using this extraction technique. Tests were conducted in four different soils with varying characteristics. The analysis showed that extractability increased for extraction solvents containing monovalent cations (K^+, NH_4^+, and Na^+) which demonstrate that cation exchange can be used as a viable technique to extract adsorbed organic chemicals from soil. The increase in extractability will allow for a more accurate evaluation of potential bioavailability and fate of organic chemicals in the environment.

MP190 Availability of arsenic and antimony in contaminated soils assessed by DGT, sequential extraction, and bioassay with water spinach (Ipomoea aquatica)

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The enrichment of soil arsenic (As) and antimony (Sb) is posing a potential threat to the environment and human health. However, the biogeochemical behavior and plant uptake of the emerging element (Sb) is poorly known and generally assumed to be similar to that of As. In this study, the lability of As and Sb under agricultural conditions in long-term contaminated soils was assessed. Three series of test soils were prepared by mixing a historically As and Sb-contaminated soil with three uncontaminated soils with varying properties. These were mixed at different ratios to produce concentration gradients. The labile As and Sb in these soils were measured by diffusive gradients in thin films (DGT, C_{DGT}), soil solution analysis (C_{sol}), and sequential extractions (SEP), which were compared with the bioaccumulation of As and Sb in different compartments of the water spinach (Ipomoea aquatica) grown in these test soils in pot experiments. Regardless of measurement techniques, the results showed that both As and Sb were less labile and strongly associated with soil solid phases, and that As was consistently more labile than Sb although total As concentrations in soils were lower than total soil Sb (e.g. 1400 mg As/kg and 2300 mg Sb/kg, respectively). The SEP showed that in historically contaminated soils, the As associated with amorphous and crystalline Fe oxides were the dominant phases, while Sb was primarily found in the residual phase. The DGT measurements revealed that the resupply of As from solid phases to soil porewater varied with soil properties, whereas the resupply of Sb was consistently very low in all soil types. The bioassay demonstrated differences in metalloid uptake mechanisms. Irrespective of soil types, more As was accumulated in roots than Sb, however As translocation from roots to shoots was lower than Sb. Correlation analysis showed that the As and Sb in plant tissues were significantly correlated with their bioavailable concentrations obtained from DGT, soil solution, and SEP, in which DGT was a better predictor for As in tissues than other measures (greater correlation coefficients). Arsenic in plant roots were more highly correlated with bioavailable As in soils than shoot uptake, the opposite was true for Sb. This study provided a greater understanding of the behavior of As and Sb and the sensitive technique for predicting bioavailable As and Sb in long-term contaminated soils to I. aquatica.

MP191 Comparison of the biogeochemical behavior of arsenic and antimony in contaminated soils and their uptake by water spinach (Ipomoea aquatica)

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The increasing input of As and Sb into the environment is putting pressure on human and environmental health. Currently there is very little known about the biogeochemical behavior of Sb and its uptake mechanisms by plants, and it is generally assumed to be the same as that of As. This insufficient data may lead to mis-interpretation of Sb behavior. In addition, their competitive effects on their uptake by plants has been poorly studied. In this study, diffusive gradients in thin films (DGT) and a sequential extraction procedure (SEP) were used to investigate the partitioning and availability of As and Sb in three series of soils amended with As only, Sb only, and mixture of As and Sb. These soils were prepared in a concentration gradient. The bioavailable As and Sb measured by DGT and SEP were compared with the bioaccumulation of As and Sb in water spinach (Ipomoea aquatica) grown in these soils in pot experiments. The results showed that the association of Sb with various soil binding sites was different from that of As and that the movement of Sb from more available to less available forms was quicker than that of As. This was illustrated by the fact that Sb was dominant in both amorphous and crystalline Fe oxides, while As was predominantly found in amorphous Fe oxide and specifically sorbed fractions. The DGT revealed that As was partially resupplied from solid phases to soil porewater, while the resupply of Sb was very low. The bioassays demonstrated the differences in As and Sb uptake mechanisms. The bioaccumulation of Sb in the plant tissues was much less than that of As due to the lower bioavailable Sb in soils. Both As and Sb were mainly accumulated in I. aquatica roots. Saturation of As uptake by I. aquatica and growth inhibition of plants grown in As treatments were observed, but these were not observed for Sb. The bioaccumulation of Sb in I. aquatica grown in soils amended with both As and Sb was much higher than that in soils amended with Sb only. As and Sb in plant tissues were highly correlated with the bioavailable As and Sb in soils measured by DGT and SEP, in which Sb uptake was better predicted. This study provided a better understanding about the behavior of As and Sb in contaminated soils and was the first to evaluate the competitive effects of As and Sb on their uptake by edible plant. These techniques are useful measures in predicting bioavailable As and Sb in recently contaminated soils to I. aquatica.

MP192 Effect of chemical speciation on the bioaccessibility of soils contaminated and spiked with heavy metals

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Heavy metal contamination continues to be a major environmental problem and current exposure guidelines set by risk assessors are based on total metal concentration. The total concentration of a potentially toxic metal in soil, while indicative of the extent of contamination, tells little about the bioaccessibility of the metal. Rather, several studies have suggested that the bioaccessibility of a metal is dependent on its chemical form, which in turn is affected by soil properties and processes. To evaluate the effect of chemical speciation on bioaccessibility, five soil types were spiked with a metal mixture comprised of copper (Cu), lead (Pb), nickel (Ni), cobalt (Co) and zinc (Zn) using three methods: metal oxides, metal nitrates plus leaching and roasted metals. Total metal concentration in each soil was determined using inductively coupled-mass spectroscopy (ICP-MS). Speciation of metals was determined using X-ray Diffraction (XRD) and X-ray Absorption Near Edge Structure (XANES) techniques. The former was used to determine metal-mineral phases, while the latter was used to determine the oxidation states of the metals. Oral bioaccessibility is being determined using the Simulator of the Human Intestinal Microbial Ecosystem (SHIME). It is expected that outer-sphere complexed metal species would be more bioaccessible than inner-sphere complexed, surface precipitated and solid phase metal species.

MP193 Spatial Contaminant Trends in a Great Lakes Area of Concern

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Sediment Samples from the Huron-Erie Corridor (HEC) (St. Clair River, Lake St. Clair and Detroit River) were collected using a stratified-random approach in a 2013-2014 survey to evaluate sediment chemistry. The results are compared and contrasted with a similar study conducted in 2004. The sediment chemistry analyses consisted of grain size, TOC, total mercury, trace metals, PCBs, PAHs and OCs. At the corridor scale there were distinct differences noted in the distribution of organic and metal contaminants. Temporal comparisons yielded distinct changes with respect to certain trace metals ($p < 0.001$) while others were shown to display no change with time. A Geographic Information System (GIS) was used to map and assess hazard index information while the Getis-Ord GI* geospatial statistic tool was used to characterize regional zones of high and low contamination for selected contaminants.

MP194 Ambient Sediment Quality Conditions in Minnesota

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Minnesota is known for its bountiful water resources, ranging from the pristine Boundary Waters Canoe Area Wilderness in northeastern Minnesota to urban waterways affected by human development. The first broad assessment of ambient sediment quality conditions has been conducted across the state. Sediment chemistry and particle size data were evaluated to provide a snapshot of the statistical range of analytes and to determine if these concentations were influenced by major watershed land uses in 54 randomly selected lakes. Data on surficial sediments included: metals and metalloids, parent and alkylated PAHs, PCB congeners, legacy organochlorine pesticides, TCDD/F congeners, PBDEs, total organic carbon, and particle size fractions. A suite of contaminants were compared to their corresponding Level I and Level II sediment quality targets (SQTs) for the protection of benthic organisms. Multivariate statistical methods were used to tease out relationships in complex data sets, and environmental forensic methods were used to identify major sources of PAHs. In addition, ambient background threshold values of contaminants were calculated for the 95% upper tolerance limit with 95% coverage. Mean probable effect concentration quotients (PEC-Qs) were calculated to distill data from a mixture of contaminants into one unitless index. Nearly 43% of lake samples had mean PEC-Qs below the Level I SQT, while the other 57% of lakes were between the Level I and II SQT values. No lakes exceeded the Level II SQT for mean PEC-Qs, for which harmful effects on benthic invertebrates would be more likely to occur. Urban lakes were more contaminated than lakes from other watershed land uses. In particular, historical uses of sodium arsenite in one urban lake contributed to arsenic concentrations that exceeded the Level II SQT value. Some metals (e.g., copper, nickel) were naturally enriched in northeastern Minnesota due to geological deposits of minerals containing these metals. Total PAH concentrations were < 1 mg/kg dry wt. in lakes outside of urban areas, and coal-related combustion and vehicle emissions were major sources. DDT metabolites were commonly detected, especially p,p'-DDE, as were PCDD/F congeners and homolog groups. Most PCDD/F toxic equivalents were between the Level I and II SQT values. BDE 209 was detected in 84% of samples. These results will be used to help prioritize agency activities related to ambient sediment quality, and for future status and trends work.

MP195 Comparative study of brine treatment using a functionalized nanofibre and an ion exchange resin

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In this study, comparative sorption studies of the major metal ions (Mg^{2+}, Ca^{2+}, K^+ and Na^+) in the brine wastewater were performed on hydrophilic materials (PAN nanofibre, PAN+TiO_2 nanofibre, PAN+ZEOLITE nanofibre) and Purolite S950 resin to investigate their uptake performances. For this purpose, PAN nanofibre was electrospun and subsequently doped with 3 wt% each of titanium dioxide and zeolite respectively, in controlled experimental conditions in order to improve its performance. This was followed by the characterization of the respective hydrophilic materials (PAN, PAN+TiO_2 and PAN+ZEOLITE nanofibres) using Fourier transform Infrared Spectroscopy (FT-IR); Scanning electron microscopy (SEM) and X-ray diffraction (XRD). SEM showed that the incorporation of titanium dioxide or zeolite into the PAN structure made the surface rougher than that of the ordinary PAN nanofibre and FT-IR revealed the peaks belonging to titanium dioxide and zeolite respectively, showing the inorganic materials are within the PAN structure. The XRD analysis complemented the FT-IR of the nanofibres by revealing the peaks characteristic of titanium dioxide and zeolite are present on the PAN structure. Batch sorption experiments were carried out to investigate the sorption properties of PAN, PAN+TiO_2 and PAN+ZEOLITE nanofibres for the cations from brine wastewater. Comparative batch studies using PAN, PAN+TiO_2 and PAN+ZEOLITE nanofibres were carried out using Purolite S950 resin as the standard. Parameters which include contact time, effect of temperature, pH, sorbent dose, sorption isotherms were studied in the sorption experiments to understand the sorption phenomena, loading capacity of the sorbents (nanofibres and resin) and the sorption kinetics for the cations from the simulated brine solutions. The results revealed that the Purolite S950 resin was effective in the removal of the divalent metal ions (Mg^{2+} and Ca^{2+}) over the other metal ions, thereby depicting good selectivity towards Mg^{2+} and Ca^{2+} ions. The removal by the Purolite S950 resin followed the order: $Mg^{2+} > Ca^{2+} > K^+ > Na^+$. The binding capacity to the divalent metal ions is influenced by the aminophosphonic acid functional group attached to the resin. The sorption capacities for the metal ions were indicating that the monolayer adsorption occurred on all the sorbents. The order of efficiency and performance of the sorbents can be given as: Purolite S950 resin > PAN+ZEOLITE > PAN+TiO_2 > PAN.

MP196 Determination of Sulfur – Containing Anions in Saline Waters using Negative-Mode Capillary Zone Electrophoresis with Indirect Detection

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Sulfides and sulfur oxyanions (i.e., thiosalts) are found in the environment and in a number of industrial processes. Sulfur oxyanions can impact environmental quality, and can have negative economic consequences. They can be reduced to the toxic and corrosive hydrogen sulfide in anaerobic environments. Whereas oxidative process on sulfide and sulfur oxyanions can lead to acidification of the environment and mobilization of toxic metals. These sulfur compounds can ultimately lead to higher operational costs whether of treatment or refit of damaged equipment. It is important to quantify the major charged sulfur species, such as sulfate SO_4^{2-}, thiosulfate $S_2O_3^{2-}$, tetrathionate $S_4O_6^{2-}$, sulfite SO_3^{2-}, and sulfide S^{2-}, to understand the chemistry and behaviour of sulfur-oxygen species as contaminants in process infrastructure. However, analysis of charged sulfur species in highly saline water is challenging. The accurate quantitation requires baseline separation and absences co-migration, which is particularly hard to achieve it due to the large peaks associated with sulfate and chloride in saline waters. A capillary zone electrophoresis (CZE) method has been developed for the simultaneous quantification of charged sulfur species in highly saline water. The components of the background electrolyte (buffers, flow modifiers, chromophoric probes, etc.) are the most important factors the development of this method. For example, pyromellitic acid (PMA) is chosen as chromophoric probe because it is non-oxidizing, has high molar absorptivity, and is a good mobility match for thiosalts, which reduces dispersion. Indirect detection using a chromophoric probe is needed because some of sulfur oxyanion species have little or no absorbance in the UV. This CZE method has been developed for negative mode, i.e. the anions migrate toward the detector, and the electroosmotic flow (EOF) is away from the detector. By adding hexamethonium hydroxide (HMOH) as an EOF modifier to influence the chemistry of the capillary surface, shorter time of analysis and better peak resolution were achieved. Other factors that were taken into consideration include: the capillary length, separation temperature, potential applied, and used of a stabilizing agent to limit spontaneous oxidation of some of sulfur-containing compounds. Fast analysis with high separation efficiency and improvement in detection sensitivity were achieved with this method. Optimization efforts and results will be presented.

MP197 Formation of Trihalomethanes (THMs) as Disinfection by-Products (DBPs) when Reclaimed Water is Disinfected with Hypochlorite

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Disinfection is an essential process in the treatment of municipal wastewater before the treated wastewater can be discharged to the environment. Hillsborough County's Northwest Regional Water Reclamation Facility (NWRWRF) in Tampa, Florida, currently uses ultraviolet (UV) light for disinfection. However, this method has proven expensive to implement and maintain, and may not be effective if the light transmission is poor. For these reasons, Hillsborough County is considering switching from UV light to sodium hypochlorite for disinfection. However, hypochlorite (chlorine) disinfection has its disadvantages as well, such as the production of disinfection by-products (DBPs) like trihalomethanes (THM) and haloacetic acids (HAAs), which may have adverse impacts on the quality of surface waters that receive the treated wastewater. Therefore, the objectives of this research are (1) to determine the chlorine demand of wastewater at NWRWRF, (2) to determine the effects of pH, temperature, and water quality on chlorine demand, (3) to quantify the DBP formation potential under different operating conditions, and (4) to determine whether NWRWRF should switch from UV light to hypochlorite for disinfection of wastewater. To inform laboratory experiments, the quality of final effluent was monitored at two nearby wastewater treatment plants that currently use hypochlorite for disinfection. At these two facilities, we observed pH of 7.0–8.0, chemical oxygen demand (COD) of 15–33 mg/L, alkalinity of 200–250 mg/L as $CaCO_3$, chlorine residual of 1.5–6.0 mg/L, and total trihalomethanes of 100–190 mg/L (mostly chloroform). Upcoming laboratory experiments will test for trihalomethane formation over incremental time periods at three different chlorine doses and three different temperatures (representing different seasonal temperatures in Tampa); results of these experiments will be reported in this presentation. Based on the experimental results, the optimal chlorine dose to disinfect the wastewater without exceeding Florida state environmental regulatory limits for THM concentrations will be determined.

MP199 An ultra-sensitive (parts-per-quadrillion) and SPE-free method for the analysis of estrogens in surface water

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Steroidal estrogens often occur in surface water at low concentrations (< 0.5 ng/L) and those low levels, depending on the estrogens present, can cause endocrine disruption in aquatic wildlife. Currently, many analytical methods are not able to detect estrogens at those low concentrations and those that can often require the extraction of several liters of water. To that end, an analytical method is presented that is sensitive to the parts-per-quadrillion (pg/L) for estrogens in surface water without the need for large sample volumes. The estrogens included for study were estrone, 17β-estradiol, estriol, 17α-ethinylestradiol, and equilin. The method consisted of the small-scale liquid-liquid extraction of surface water (7.5 mL) followed by derivation with dansyl chloride. Analyte separation and detection were performed by high-performance liquid-chromatography and tandem mass-spectrometry. A large volume (100 uL) of the sample was injected on-column to increase the analyte mass sent to the detector. The detection limits of the method were 0.045 ng/L for estrone, 0.086 ng/L for 17β-estradiol, 0.030 ng/L for estriol, 0.049 ng/L for 17α-ethinylestradiol, and 0.13 ng/L for equilin. The whole-method accuracy ranged from 93 ± 5.8 % to 105 ± 4.5 % for all the analytes at two different spike levels. Similarly, the precision of the method was less than 8.0 % relative standard deviation. The final method was used to analyze a series of samples from the Mississippi River spanning 51 river miles. Estrone was detected in all of the samples and 17β-estradiol was detected in one. Concentrations of estrone ranged from between the detection and quantification limits up to 0.63 ng/L. Increases in the concentration of estrogens were observed downstream from potential inputs including wastewater and drinking water treatment plants. This work sugests that drinking water treatment plants can also act as sources of micro-pollutatnts. Additional data and/or applications of this method will be presented as they become avalable.

MP200 Modeling Singlet Oxygen Production by Dissolved Organic Matter

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Photodegradation can be an important fate of aquatic contaminants. One route is direct photolysis, where the contaminant absorbs light and subsequently degrades. Many contaminants, though, do not absorb at solar wavelengths. In these cases indirect photolysis may occur via reaction with species generated by a photosensitizer. Among the natural photosensitizers, dissolved organic matter (DOM) is the most ubiquitous, and it produces a number of reactive oxygen species including hydroxyl radical, superoxide, and singlet oxygen ($^1O_2$). $^1O_2$ is of interest because it reacts with several important classes of compounds including phenolates, furans, indoles, and imidazoles. $^1O_2$ will exist in a steady-state as it is formed by DOM and simultaneously relaxes via quenching by water. Because of this, reaction of $^1O_2$ with contaminants follows pseudo first-order kinetics, and the rates can be modeled if the steady-state concentration is known. To predict $^1O_2$ steady-state concentrations several parameters must be known, including the spectral distribution and intensity of sunlight, the absorption spectrum of DOM in the water of interest, and the $^1O_2$ quantum yield (Φ_{1O2}) as well as its wavelength dependence. While the light intensity and DOM absorption are relatively easy to measure, it is challenging and time consuming to measure the Φ_{1O2} wavelength dependence, and it is impractical to measure regularly, as water quality and DOM reactivity change seasonally and spatially. Here, we explore the merits of a simplified modeling approach based on previous work[1-4] showing a correlation between Φ_{1O2} at 365nm and DOM E2/E3 ratios, the ratio of absorption at 254nm and 365nm. Using several DOM samples, we will use Φ_{1O2} predicted from E2/E3 ratios to calculate $^1O_2$ concentrations for comparison with experimental values during irradiation with a broadband solar simulator. A variety of calculation approaches are being explored to define the level of spectral detail required to adapt single wavelength quantum yields for modeling $^1O_2$ concentrations with broadband solar irradiation. Critical comparisons of traditional full-spectrum modeling with simplified calculation approaches will then be presented. References 1. Dalrymple et al. Environ. Sci. Technol. 2010, 44, 5824-9 2. Mostafa et al. Environ. Sci. Technol. 2013, 47, 8179-86 3. Peterson et al. Environ. Sci. Technol. 2013, 46, 7222-9 4. Bodhipaksha et al. Environ. Sci. Technol. 2015, 49, 3453-63

MP201 An analytical method for the determination of emerging contaminants in water samples from Brazilian rivers

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The contamination of the aquatic environment by emerging pollutants such as pharmaceuticals, personal care products and endocrine disruptors has been the focus of recent research due to their potential hazard to human health and the environment. These compounds can enter the environment by direct or indirect routes. They accumulate in soil, sediments and river water, and can be transported over long distances from their sources. Suitable analytical methods are therefore needed to investigate the occurrence and transport of these pollutants in the environment. The purpose of this research was to create an analytical method for the extraction and detection of emerging contaminants in river water samples. Chromatography analysis was carried out using an LC – DAD. The results were satisfactory with a flow rate of 0.8 mL min^{-1}, temperature of 25 °C and a mobile phase of acetonitrile : water with 0.1% of acetic acid. For isolation of the target compounds from the matrix solid phase extraction was applied. The optimization of the extraction procedure was evaluated. With the proposed method it is possible to quantify acetaminophen, methyl paraben, 17β-estradiol, naproxen and diclofenac at concentrations of 10 μg L^{-1} and 20 μg L^{-1} for 17β-estradiol. The method developed will be applied to water samples from the Mogi Guaçu River in the state of São Paulo, Brazil.

MP202 Temporal Changes in Detection Limits of Pesticides in Surface Water

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Stantec Consulting Ltd. (Stantec) investigated possible temporal changes in environmental detection limits (i.e., method detection limits (MDL), limits of quantification (LOQ) and reporting detection limits (RDL)) for pesticides and how, if any, these changes need to be communicated in the context of toxicological risk assessment. The study focused on three conceptual questions: 1) How are pesticides detected in environmental monitoring programs and how have pesticide detection limits changed over time? 2) What effect does the detection limit have on the frequency of detection? 3) How do the changes in detection limits affect the interpretation of risk in terms of exposure of ecological receptors (e.g., fish, invertebrates, aquatic plants) to pesticides? The study included a multiple lines of evidence approach that considered data from an accredited analytical laboratory in Canada; data reported in published peer-reviewed scientific literature; and 25 years of data obtained from a comprehensive surface water monitoring program in California that involved multiple laboratories and agencies. As expected, the records from the analytical laboratory indicated that changes in detection techniques or improvements in existing techniques have resulted in a decrease in detection limits over time. Regardless of the ability to detect pesticides at lower concentrations, results from the scientific literature and from the surface water monitoring program suggest there is no discernable downward trend in pesticide detection limits in surface water between 1990 and 2015. In fact, the literature indicates that there has been an increase in the reported detection limits for some pesticides. This trend is likely related to use of novel techniques that focus on cost and portability, rather than low detection limits. Analysis of the monitoring program data indicates that the detection limit can have a substantial effect on the frequency of detection and confirmed that the lower the detection limit the higher the frequency of detection. However, a higher frequency of detection does not necessarily translate into increased risk for aquatic receptors. Measured concentrations, not simply presence or absence, need to be compared to environmental quality guidelines to make inferences about potential risks to receptors.

MP204 Improved Sample Preparation for the Determination of Persistent Organic Pollutants in Human Milk

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Although they were banned in 1970s and 2004, respectively, polychlorinated biphenyls (PCBs); organochlorine pesticides (OCPs), and polybrominated diphenyl ethers (PBDEs) are still ubiquitous in the environment and pose adverse health effects in humans and wildlife. Therefore, it was necessary to improve our outdated analytical method for complex matrices like breastmilk that was time consuming, labor intensive and required large volume of solvent usage. We have developed an improved sample preparation method that combines liquid-liquid extraction and automated solid phase clean-up procedures using only 1 mL of milk sample for the analysis of PBDEs, PCBs and OCPs by high resolution GC/MS. This new method improves throughput (minimum of 40 samples per batch run); significantly reduces the solvent amount from 400 mL to 25 mL; shortens the sample preparation time from 5 days to 3 days; has good precision (≤ 10% coefficient of variation for most of the compounds measured) and accuracy (± 20% recoveries for most of the compounds measured in in-house and SRM1954 samples); and reduces background contamination while providing a lower method detection limit. This method has been validated using fortified in-house quality control samples, a standard reference material (SRM1954), as well as fifteen breast milk samples from an epidemiological study with high, medium, and low levels of PBDEs, PCBs, OCPs measured previously using the traditional method. Levels of PBDEs, PCBs and OCPs from the two methods showed acceptable consistencies. The views expressed herein are those of the authors and do not necessarily reflect those of the California Department of Toxic Substances Control.

MP205 Levels of Blood Organophosphorus Flame Retardants and Association with Changes in Human Sphingolipid Homeostasis

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While a recent toxicological study has shown that organophosphorus flame retardants (OPFRs) may disrupt sphingolipid homeostasis, which plays an essential role in key physiological and pathological processes, epidemiologic evidence is currently lacking. In this study, a total of 257 participants were recruited from Shenzhen, China. Eleven OPFRs were for the first time simultaneously determined in the human blood samples by ultraperformance liquid chromatography tandem mass spectrometry. Six OPFRs, tributyl phosphate (TBP), 2-ethylhexyl diphenyl phosphate (EHDPP), tris(2-chloroisopropyl) phosphate (TCPP), tris(2-butoxyethyl) phosphate (TBEP), triethyl phosphate (TEP), and TPhP were detectable in at least 90% of participants, with the median concentrations of 37.8, 1.22, 0.71, 0.54, 0.49, and 0.43 ng/mL, respectively. Sphingomyelin (SM) levels in the highest quartile of EHDPP, TPhP, TBP, TBEP, TEP and TCPP were 45.3% [95% confidence interval (CI): 38.1%, 53.0%; $p < 0.001$]; 51.9% (95% CI: 45.5%, 58.6%; $p < 0.001$); 153.6% (95% CI: 145.1%, 162.3%; $p < 0.001$); 20.6% (95% CI: 14.5%, 27.0%; $p < 0.001$); 59.0% (95% CI: 52.1%, 66.2%; $p < 0.001$) and 62.8% (95% CI: 55.2%, 70.6%; $p < 0.001$) higher than those in the lowest quartile, respectively, after adjusting for covariates. Sphingosine-1-phosphate (S1P) levels in the highest quartile of EHDPP, TPhP and TBP were 36% (95% CI: -39%, -33%; $p < 0.001$), 16% (95% CI: -19%, -14%; $p < 0.001$) and 36% lower (95% CI: -38%, -33%; $p < 0.001$) than those in the lowest quartile, respectively. A similar pattern emerged when exposures were modeled continuously. We for the first time found the evidence that OPFRs might affect sphingolipid homeostasis in humans.

MP206 Concentrations of Brominated Dibenzo-Dioxins and Furans in Dust from Residences and the Living Quarters of Firehouses

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Large reservoirs of polybrominated diphenyl ethers (PBDEs) still exist in the United States, even after their production and use have been discontinued. One such reservoir of PBDEs is dust from residential and occupational settings. Previous studies in our group (2010, 2010-2011) have shown high levels of PBDEs in dust from homes and from the living quarters of firehouses in California, collected from vacuum cleaner bags. These studies showed higher concentrations of PBDEs in the firehouse dust than residential dust. Thermal and photodegradation of PBDEs can form polybrominated dibenzo-dioxins and furans (PBDD/Fs) which exhibit similar toxicity to their chlorinated analogs. Thus, exposure to dust that is high in PBDEs may also result in exposure to potentially significant dioxin toxicity. Our group has expanded on these studies of residential and firehouse dust to include PBDDs/Fs. However, dust can be a difficult matrix to work with. Despite their similar structures and toxicity, the cleanup method that we use for chlorinated dioxins and furans does not work for PBDDs/Fs. Therefore, we applied a modified version of the chlorinated dioxin/furan cleanup method to the cleanup of dust samples for PBDDs/Fs. In this modified method, dust samples were extracted using pressurized fluid extraction. Cleanup was accomplished by eluting the concentrated extract through a mixed mode silica gel column, followed by elution through a mixed-mode carbon column in two separate steps, rather than in one combined step as is done with the unmodified method. Samples were analyzed using high resolution GC/MS. For PBDDs in both residential and firehouse dust samples, no 2,3,7,8-susbtituted PBDDs were detected, and of the non-2,3,7,8-substituted PBDDs, only HpBDD was measured at 16.8 and 24.5pg/g, respectively. For 2,3,7,8-substituted PBDFs in residential dust, only 1,2,3,4,7,8,9-HpBDF was detected at high concentration (424 pg/g). For firehouse dust, 2,4,6,8-TeBDF, 1,2,4,4,7,8-HxBDF, and 1,2,3,4,7,8,9-HpBDF were detected at 714 pg/g, 259 pg/g, and 6,100 pg/g, respectively. For total PBDFs in residential dust, only HpBDF was measured at high concentration (514 pg/g). For total PBDFs in firehouse dust, we measured TeBDF (1050 pg/g), PeBDF (213 pg/g), HxBDF (584 pg/g, and HpBDF (10,800 pg/g). The views expressed herein are those of the authors and do not necessarily reflect those of the California Department of Toxic Substances Control.

MP207 Simplifying Multivariate Statistical Tools: Case Study of Dioxin/Furan Congener Profiles at a Former Wood Processing Site in Puget Sound, WA

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Environmental data sets are complex and highly variable. Multivariate statistical models have long been used to help reduce this variability. However, the outputs of these models are often complex in their own right, requiring discussions about statistics that only further confound, rather than inform, public and regulatory audiences. When applied correctly, data visualization techniques, including simple X/Y graphs and Geographical Inromation Systems (GIS), can be used to display the model results in ways that are both descriptive and intuitive. NewFields has applied a variety of multivariate statistical methods to better explain patterns in data from contaminated sediment sites across the country. A particular focus has been characterizing dioxin/furan congener profiles at wood processing sites in Puget Sound. One of these sites in Budd Inlet (Olympia), WA, will be used as a case study for combining multivariate statistics and visualization techniques to explain patterns in contamination. Several hundred dixon/furan samples have been collected from Budd Inlet over the last decade. Multivariate statistical modeling revealed that three different end-members (unique dioxin/furan congener profiles) contributed nearly 98 percent of the total variance in congener profiles across the Inlet. The three end-members correlated strongly with hog fuel boiler emissions, pentachlorophenol, and polychlorinated biphenyls (PCBs). Nearly all samples represented a mixture of these end-members, making interpretation of the model more difficult. To reduce noise, only samples dominated by one of the end-members were mapped, revealing distinct spatial patterns for each of the three potential sources. These patterns were linked to potential upland pathways, providing a comprehensive link between upland and sediment contamination.

MP208 Estimating detection levels for multi-analyte methods: beyond the EPA MDL procedure

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The U.S. Environmental Protection Agency's method detection limit (USEPA MDL) procedure often is mandated when USEPA methods are used or for studies conducted for federal, state, or local regulatory agencies. The MDL procedure has several well documented limitations, and procedural revisions have been proposed that address some limitations (Federal Register, v. 80, no. 33, p. 9074). One limitation is the requirement that a cumbersome iterative spiking approach be used to ensure that the analyte concentration in spiked test samples is within 1-5 times the determined MDL, as spiking level influences the calculated MDL. This approach is impractical, in part, because it is difficult and expensive to create a solution of analytes, each at the appropriate concentration, for methods with many analytes having differing instrument-response characteristics. Instead, the USGS National Water Quality Laboratory (NWQL) has implemented aspects of ASTM International's within-lab critical level (WCL) estimate procedure (D7782-13) and associated Excel®-based DQCALC® calculator for estimating detection levels (DLs). The WCL procedure uses a multi-concentration (calibration-like) spiking approach well suited for analytes having very different instrumental response, and models the change in standard deviation with spike concentration. It estimates both the MDL and Currie's critical level (Lc), which in theory should closely approximate the MDL value. The NWQL has applied the WCL procedure to inorganic (e.g., nutrients, trace metals) and organic methods (e.g., a direct aqueous injection LC/MS/MS method for 227 pesticides in filtered water having no sample preparation steps and a solid-phase extraction GC/MS method for 83 pesticides with prep steps) to demonstrate the operational advantage of using the multi-concentration spiking procedure. For MS methods, DLs estimated by either procedure using the quantification ion (concentration) were substantially lower than the "true" DL for some analytes because qualification ion responses were too low to confirm analyte presence at the DL. For blank-limited analytes, DLs also were calculated using lab blank data that provide a more accurate, direct estimate compared to those from spike-based procedures, which can be artificially low. Advantages and limitations of the WCL procedure are presented. Any use of trade, product, or firm names is for descriptive purposes only and does not imply endorsement by the US Government.

21st Century Approaches for Cross-Species Extrapolation in Toxicity Assessment

MP209 Characterizing molecular toxicity pathways of selected emerging contaminants to elucidate species-specific sensitivity of three North American fishes

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Emerging chemical contaminants (ECC) are ubiquitous in the environment, and have become of increasing toxicological concern to humans and wildlife. ECCs include pharmaceuticals and personal care products, brominated flame retardants, and nanomaterials, among others, which are commonly discharged into surface waters through municipal wastewater and other sources as a result of human activities. However, little is known about the toxicological significance of most ECCs in the aquatic environment. Particularly, little is known about the effects of ECCs to fishes that are of commercial, cultural, and recreational relevance (CRA species) to North America and that are at risk of exposure with ECCs. Therefore, this study aims to investigate the responses and the underlying mechanisms of the exposure to ECCs of three CRA species including lake trout (Salvelinus namaycush), white sturgeon (Acipenser transmontanus), and rainbow trout (Oncorhynchus mykiss). Specifically, the objectives of this study are to (1) characterize critical molecular toxicity initiating events (MIEs) associated with each ECC; (2) assess the conservation of gene expression signatures across fish species; and (3) construct ECC-induced molecular toxicity pathways, and anchor these to apical outcomes. Fishes at early life stages will be exposed to three representative ECCs, namely nanosilver (AgNP), 17α-ethinylestradiol (EE2), or fluoxetine (Prozac). Whole transcriptomic and proteomic coupled with receptor-binding, cellular and biochemical assays will be used to characterize critical toxicity pathways. Finally, mechanistic information from molecular toxicity pathways will be linked with apical responses across higher tier biological organizations. Linking results from open omics analyses with effects at higher levels of biological organization aims to identify and establish relevant biomarkers for environmental risk assessment.

MP210 Determination of Acute and Sub-Chronic Toxicity of Emerging Contaminants in Early Life Stages of Three Canadian Fish Species

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In recent years, emerging contaminants have gained notoriety due to their ubiquity in the aquatic environment as well as the lack of data available regarding their toxicity to wildlife and humans. Emerging chemicals (ECs) of concern such as hexabromocyclododecane (HBCD), silver nanoparticles (AgNPs), short-chain chlorinated paraffins (SCCP), 17α-ethynylestradiol (EE2) and Prozac™ (FLX) primarily enter the aquatic environment as mixtures through municipal wastewater effluent (MWWE). MWWE, which is typically a mixture of industrial, commercial, and household wastes, may be released into receiving waters with little to no treatment, which is not uncommon, especially in rural Canadian municipalities. Most data to date has been garnered using standard laboratory species, which may not be particularly relevant to Northern species considering the potential role of life history, trophic level, physiology, and climate on the species-specific toxicity of chemicals. Consequently, inaccurate extrapolation from standard laboratory species to species native to northern ecosystems is a cause for concern and represents a significant uncertainty factor in ecological risk assessment. In this study, Oncorhynchus mykiss, Salvelinus namaycush, and Esox lucius gametes were exposed to six waterborne concentrations of EE2, FLX, MWWE, and AgNPs, where the lowest doses were selected based on environmental relevance and increased incrementally thereafter. Exposures were continuous flow-through and subsamples were collected at critical developmental stages to assess acute and sub-chronic toxicity of all test chemicals. Initial findings suggest that these three species vary significantly in their sensitivities towards the aforementioned ECs. Ongoing work aims to fully elucidate biochemical and histological anomalies associated with exposures to the six ECs and focuses on characterizing the effects of these ECs on native fish species in comparison to one another as well as standard laboratory fish models. Overall, this work will aid in the development of more appropriate environmental risk assessment strategies for native fishes to EC of concern.

MP211 Lower sensitivity of cyprinid fishes to three acetylcholinesterase inhibitor pesticides: an evaluation based on no effect concentrations

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Cyprinid fishes are often hypothesized to be less sensitive to chemical stress than other comparable fish species, yet a decisive empirically based evaluation of this hypothesis seems to be lacking from the literature. To fill this gap, we developed a generalized linear mixed model in which no effect concentrations (NEC) of 29 fish species from 14 families exposed to one of three acetylcholinesterase inhibitor pesticides (carbaryl, chlorpyriphos, and malathion) were used as a response variable, whereas (i) the corresponding specific somatic maintenance (SSM) rates and (ii) a categorical variable if a species is Cyprinidae were used as the predictor variables. We included SSM rates in the analysis because a previous study had demonstrated that the SSM rates negatively correlate with the NECs. Our results indicated that the NECs for cyprinid fishes were significantly higher than those for other fishes, suggesting that cyprinids are less sensitive to the three studied pesticides. The SSM rates were negatively related with the NECs, but the actual relationship between the two was not clear, implying that the importance of SSM rates may depend on a taxonomic group tested.

MP212 Comparative behavioral response patterns of two common larval fish models

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Behavioral responses inform toxicology studies by rapidly and sensitively reflecting molecular initiation events that propagate to physiological changes in individuals. These behavioral response patterns can be linked to a chemical mechanism/mode of action (MOA). For example, behavioral “fingerprints” have been proposed following pharmacological screening studies with zebrafish, which are common models in behavior, developmental, neurotoxicology and other biomedical studies. Whereas the fathead minnow is a common model for aquatic toxicology research and regulatory programs, they have received comparatively little attention in behavioral studies. We employed the zebrafish and fathead minnow models to define toxicant induced swimming activity alterations during interchanging photoperiods. We specifically compared behavioral response patterns among 14 chemicals, each classified according to its anticipated MOA following QSARs developed to predict toxicity to aquatic organisms. The classes consisted of non-polar narcosis (class I), polar narcosis (class II), electrophile/proelectrophile activity (class III), and specifically acting (class IV). Following OECD FET and EPA WET experimental guidelines, zebrafish embryos and fathead minnow larvae were exposed for 96 h to each compound, then observed using a digital behavioral analysis system (ViewPoint). Behavioral observations occurred for 50 minutes (10 minutes acclimation, two 10 minute dark periods, two 10 minute light periods). Zebrafish displayed greater behavioral sensitivities for Bisphenol-A, citalopram, cumenhydroperoxide, dinoseb, hydroquinone, pentylenetetrazole, phenol and perfluorooctanoic acid, whereas the fathead minnow was more sensitive to 1-heptanol, diazinon, indene, r-carvone, tert-butyl hydroperoxide, and xylazine. Additionally, each species displayed different responses to altering light and dark photoperiods. Zebrafish responded to light photoperiods by significantly decreasing swimming activity, whereas fathead minnow displayed an opposite response. Furthermore, each of the compounds produced larval swimming patterns that appear unique to their respective classification. Behavioral studies such as these are ongoing to further characterize fish behavior patterns to diverse industrial chemicals for which behavioral responses and specific molecular initiation events are poorly defined in fish.

MP213 Use of White Sturgeon Data in Development of EPA's National 304(a) Chronic Selenium Criterion for the Protection of Freshwater Aquatic Life

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The U.S. Environmental Protection Agency (EPA) recently updated its chronic aquatic life criterion for selenium to reflect the latest science based on fish reproductive toxicity data, including key data for the white sturgeon (Acipenser transmontanus). A white sturgeon maternal transfer study (Linville, 2006) was evaluated by EPA and incorporated into the aquatic life criterion analysis. Survival and observations of abnormalities (edema, skeletal deformities) from the oldest stage (45) were quantitatively used to derive the EC10 used in the effects analysis for the criterion because these endpoints showed the most significant effect. The resulting EC10 demonstrates that white sturgeon are among the most sensitive freshwater fish species to selenium, based on available data. The white sturgeon is a commercially and recreationally important fish species in the Pacific Northwest, serves as a surrogate for other sturgeon species in the United States, and has a population listed as endangered in the Kootenai River in Idaho and Montana. EPA's 2016 freshwater criterion for selenium is composed of four elements: two fish tissue elements, and two water elements. All four elements are based on reproductive effects in freshwater fish as the assessment endpoint. The EPA derived the water-column values from the egg-ovary value by assessing food chain bioaccumulation at representative field sites across the continental United States.

MP214 The Allometric Relationship Between Acute Toxicity and Individual Body Weight for Fishes Based on Biotic Ligand Toxicity Model :A Case Study as Zinc

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Prediction of lethal effect on organism of interest that lacks of test data is critical for risk assessment of chemicals on aquatic ecosystems. The author considered body weight of organism plays an important role in counteracting toxic stress imposed by certain chemical. The zinc acute toxicity data for adult or sub-adult fishes were selected form the ECOTOX database. In light of the knowledge that test circumstances such as Ca^{2+}, hardness may throw remarkable influence on the observed toxicity. The author checked and retrieved all details for every record. The acute toxicity data such as 96-hr LC_{50} (18 data) and x-hr LC_{50} (46 data) values of fishes were correlated to corresponding body weights respectively. It showed us that the 96-hr LC_{50} and x-hr LC_{50} both have a significant correlation with body weight (R^2=0.19, 0.38; p-value=0.08, 6.1×10^{-6}). In order to explain the relationship between the acute toxicity and body weight, the author built A Biotic Ligand Toxicity Model (BLTM) based on A Biotic Ligand Model (BLM) which taking competing metal ions such as Ca^{2+} into account. The BLTM with only 4 parameters of no-effect concentration (NEC), killing rate (K_+), maximum elimination rate (K_{gamax}) , and elimination arrearage constant (Beta) were employed to model the Zn 96-hr and x-hr LC_{50} of fishes. Presumably the NEC, Beta, K_+ or K_{gamax} was each modeled to be independent of the body weight by a linear relationship. For the presumed size-dependency, optimization was performed, and finally the "best" model was selected out of the total combinations of size-dependency using the Akaike Information Criterion (AIC). The selected best model indicated that the K_+ and K_{gamax} has a good correlation with body weight whereas the NEC, Beta did not. It shows that NEC=0, Beta=196.0 (1/h), K_+=0.040*log(W) (hl/mg), K_{gamax}=23.4*log(W) (1/h), in which W denotes fish body weight (wet weight, mg). Based on the best model, the exposure time and the weight of fishes were applied to predict the fraction of survival according to BLTM. The predicting survival fractions are in the vicinity of 0.5 for both 18 and 46 data. The author considered the optimized size-dependence of the acute ecotoxicity data across fishes will be helpful for ecotoxicity data prediction and ecological risk assessment. The further research will pay more attention to the nonlinear relationship between the parameters and body weight.

MP215 Comparing toxin-induced cardiac defects in Atlantic sturgeon with those in zebrafish: A novel mechanism of AhR function in PCB-exposed fish

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Polychlorinated biphenyls (PCBs) and 2, 3, 7, 8-Tetrachlorodibenzodioxin (TCDD) are significant environmental hazards in waterways and soils across the world. These toxins cause a variety of developmental defects in exposed organisms, including severe cardiac defects in fish. When TCDD and coplanar PCBs, including PCB 126, enter the cell they bind to the aryl hydrocarbon receptor, AhR, eventually instigating gene expression of cyp1A. Although cyp1A is expressed in cells with activated AhR, cyp1A does not appear responsible for cardiac or endothelial abnormalities in exposed animals, suggesting an alternate pathway as the cause of these defects. We are identifying the molecular basis of cardiac phenotypes in fish embryos by exploring other proteins activated by AhR. Activated AhR is known to up-regulate many cellular proteins, including tyrosine kinase Src. Activated Src is further known to phosphorylate vascular endothelial cadherin (VE-cadherin), an intercellular junction protein found in cardiovascular endothelial cells. VE-cadherin functions to bind neighboring cells and inappropriate phosphorylation results in the dissociation of intercellular dimers, separating the cells. We hypothesize that up-regulation of Src, by coplanar PCB- and TCDD-activated AhR, leads to VE-cadherin phosphorylation. Thus, PCB 126 and TCDD exposure likely leads to decreased ability of vascular cells to bind appropriately, resulting in organ-level deformities of the heart during important stages of development. Utilizing contaminants at varying concentrations, we examined the effects on heart development of early exposure in Atlantic sturgeon (Acipenser oxyrinchus oxyrinchus) and zebrafish (Danio rerio). Embryos of both species exposed to PCB 126 and TCDD show very similar cardiac looping defects in a dose-dependent manner. These data suggest highly conserved mechanisms of heart development and responses to toxin exposure. Our evaluation of the cellular mechanism of PCBs using zebrafish provides a roadmap for assessing these responses more broadly in other taxa and we expect parallels in Atlantic sturgeon.

MP216 High conservation in transcriptomic and proteomic response of white sturgeon to equipotent concentrations of 2,3,7,8-TCDD, PCB 77, and benzo[a]pyrene

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Adverse effects associated with exposure to dioxin-like compounds (DLCs) are mediated primarily through activation of the aryl hydrocarbon receptor (AHR). However, little is known about the cascades of events that link activation of the AHR to apical adverse effects. Therefore, this study used high-throughput, next-generation molecular tools to investigate similarities and differences in whole transcriptome and whole proteome responses to equipotent concentrations of three agonists of the AHR, 2,3,7,8-TCDD, PCB 77, and benzo[a]pyrene, in livers of a non-model fish, the white sturgeon (Acipenser transmontanus). A total of 926 and 658 unique transcripts were up- and down-regulated, respectively, by one or more of the three chemicals. Of the transcripts shared by responses to all three chemicals, 85% of up-regulated transcripts and 75% of down-regulated transcripts had the same magnitude of response. A total of 290 and 110 unique proteins were up- and down-regulated, respectively, by one or more of the three chemicals. Of the proteins shared by responses to all three chemicals, 70% of up-regulated proteins and 48% of down-regulated proteins had the same magnitude of response. Among treatments there was 68% similarity between the global transcriptome and global proteome. Pathway analysis revealed that perturbed physiological processes were indistinguishable between equipotent concentrations of the three chemicals. The results of this study contribute towards more completely describing adverse outcome pathways associated with activation of the AHR.

MP217 Functional Genomic Screening Using CRISPR-Cas9 Knockout System in Human Cells Identifies Genes Involved in Toxicant Susceptibility

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Genome-wide functional screening is a powerful approach to study cellular responses to toxicants. The CRISPR-Cas9 genome-wide knockout system has emerged as a robust tool for loss-of-function (LoF) screening in mammalian cells. In functional toxicogenomics, this system can be used to identify genes whose functions can modulate the toxic effect of a compound. The absence of LoF genetic screening platforms in fish cells necessitates identification of toxicant susceptibility genes in mammalian cells and extrapolation of the findings to fish orthologs. We successfully performed loss-of-function screens in K562, a human erythroleukemic cell line, to identify cellular components affecting sensitivity to certain chemicals. We used a genome-wide CRISPR-Cas9 knockout library (GeCKOv2, library A) that utilizes 65,383 guide RNAs (gRNAs) targeting 19,050 human protein-coding genes and 1,864 miRNA genes. The library constructs were delivered to cells using lentiviral transduction to generate a heterogeneous population of mutant K562 cells with a single targeted gene per cell. The mutant cells were exposed to a sub-lethal dose of each toxicant in addition to the corresponding control conditions for ~ 2 weeks. At the end of the screen, the abundance of a specific mutant in each condition was determined by quantifying the corresponding guide sequence using next generation sequencing. We identified novel genes whose inactivation resulted in increased sensitivity/ resistance to each of the studied toxicants. To assess whether similar toxicity pathways are utilized across species, orthologs of genes identified in our screens can be individually knocked down in zebrafish followed by exposure studies using the same toxicants. Our work further demonstrates the strength of high throughput genetic screening using the CRISPR-Cas9 system in deciphering mechanisms of toxicity and could set a platform to study commonalities and differences in toxicity mechanisms across different species.

MP218 Coral Health Biomarker Studies using Cultured Corals and Field collected Larvae (Montipora capitata) exposed to pyrene, benzene, and gasoline

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Coral reef health is a global concern and characterization of potential anthropogenic impacts requires sensitive indicators. Therefore, rapid and non-destructive methods to assess the health of corals and their symbiotic algae (zooxanthellae) are needed or management and conservation of coral ecosystems. This study evaluated the utility of Fluorescence Induction and Relaxation (FIRe) measurements in field and laboratory studies for monitoring and assessing coral health. Phase I of this study examined three coral species Acropora divaricata, Montipora capricornis and Caulastrea furcata exposed to 50 and 200 ppb Cu,10 and 100 ppb pyrene, or elevated temperature (24-28 C) for 72 hours. FIRe results were compared to measurements of lipid peroxidation and glutathione. Phase II of this study used Montipora capitata larvae bundles collected at the Hawaii Institute of Marine Biology and Pearl Harbor in Oahu, Hawaii. The larvae bundles were exposed to water-soluble fractions of benzene and gasoline for 10 hours. Following FIRe measurements, larvae were analyzed for lipid peroxidation (MDA), glutathione (GSH), glutathione s-transferase (GST), superoxide dismutase (SOD) and ethoxyresorufin o-deethylase (EROD). Biochemical markers detected stress only at the highest contaminant concentrations, while FIRe measurements appeared more sensitive at lower exposure levels. Such integrated assessments using complimentary techniques provide useful data to better understand responses of corals to multiple environmental stressors.

MP219 Phytoremediation potential of some plants growing wildly on polluted soil and water

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The study investigates “the use of plants for the remediation of metal-polluted soil sediment, andwater. Four plant samples and two water samples were collected from a canal located at Mesan, Ogun State.The samples were stored in polythene bags and labelled appropriately. The soil samples were collected with the aid of soil auger and air-dried for about one week. The soil samples were sieved while plant samples were pulverized prior to analysis. The heavy metal concentration in the plant, water and soil were determined by the use of the Atomic Absorption Spectrophotometer. The results revealed that Heliotropiumindicum ($D_{L.P}$) accumulated more lead (35mg/g) than other land plants, Amaranthusspinosus ($B_{L.P}$)accumulatedhigh levels of copper (47.5mg/g) and lead (32.1mg/g), Cyperussp ($D_{W.P}$) accumulated high levels of copper (23.2mg/g) and lead (21.4mg/g), Cyclosorusstriatus ($C_{W\text{-}P}$) accumulated high levels of zinc (33mg/g) and Nymphaea Lotus ($A_{W\text{-}P}$) accumulated high levels of copper (20mg/g), chromium (11mg/g) and fairly moderate levels of zinc (12.1mg/g).From the results, it is seen thathyperaccumulating plants could be useful in phytoremediation of contaminated environment. The study concluded that plants that hyper accumulate metals have tremendous potential for application in remediation of metals in the environment.

Hydraulic Fracturing Operations and Issues

MP220 Do air pollutants associated with unconventional oil and gas extraction have endocrine activity?

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In the last decade unconventional oil and gas (UOG) extraction has rapidly proliferated throughout the US and the world. This has occurred largely because of the development of directional drilling and hydraulic fracturing which allows access to fossil fuels from geologic formations that were previously not cost effective to pursue. Due to the locations of formations of interest, sites of UOG activity are increasingly in greater proximity to highly populated areas. This technology is known to use greater than 1000 chemicals such as solvents, surfactants, detergents, and biocides. Further this process also releases a complex mixture of chemicals from the formations including heavy metals, and naturally-occurring radioactive and organic compounds many of which are volatile. Chemicals detected in water near UOG operations have been shown to have endocrine activity. Further recent studies have shown compounds associated with UOG activity to be linked to adverse reproductive and developmental outcomes in humans and laboratory animal models. This study aims to 1) identify air pollutants detected near UOG activity and 2) evaluate the potential endocrine activity of the most frequently detected air pollutants. Using PubMed and Web of Science, studies that measured air pollutants associated with sites of UOG in the US were identified. Titles and abstracts were screened for relevance using pre-determined inclusion criteria. Relevant articles were then reviewed in full to generate a list of the most frequently detected compounds. The most frequent compounds were then assessed for potential endocrine activity by searching PubMed and Web of Science for primary studies that evaluated them for potential estrogenic, androgenic, and thyroidogenic activity. This report summarizes the data from studies measuring air pollutants near sites of UOG including variables such as distance from the UOG site(s), number of studies that detected the compound, and the average and range of concentrations detected from each study. It also describes the endocrine activity of air pollutants most frequently linked to UOG operations. The results of this study provide a basis for prioritizing future research on health impacts associated with UOG activity.

MP221 Ecological Risk Assessment of Coal Seam Gas Hydraulic Fracturing Fluids in Australia

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Coal seam gas (CSG) production in Queensland, Australia, requires a quantitative ecological risk assessment (QERA) as part of the requirements of the Environmental Authority and the Environmental Impact Statement. The QERA process is important in informing the risk manager of the necessary risk information to cost effectively comply with the requirements of the Environmental Authority. This methodology, incorporating best practice national or international standards and guidelines, includes the identification of the hazards of the constituents in the flowback water, compilation of the most appropriate toxicity criteria for each constituent, identification of potential receptors and completed (and significant) exposure pathways, quantification of exposure (expressed as a daily intake or exposure concentration), and calculations of individual constituent risk for potentially complete exposure pathways for each ecological receptor. The conceptual site model for the hydraulic fracturing flowback water identified both terrestrial (kangaroo, Dingo, beef cattle), and aquatic (invertebrates and fishes) ecological receptors that may be exposed to hydraulic fracturing fluids either stored within ponds in the operational areas, or inadvertently released to surface water ecosystems located near the well pads. The QERA estimated potential exposures based on theoretical (mass balance of fracturing chemicals used) and empirical (analytical sampling) concentrations in the flowback water. Biodegradation rates and varied flowback dilution events were used to provide a range of exposure scenarios. The selected assessment endpoints included survival and reproduction of beef cattle, kangaroo, Dingo, and aquatic invertebrates and fish. Because of the proprietary nature of the hydraulic fracturing chemicals, toxicity reference values (TRVs) were developed as predicted no-effects concentrations (PNECs) for aquatic receptors, and body weight scaling TRVs were developed for terrestrial receptors based on applicable effects levels. Limited risks to cattle and native mammals were identified, and only in the most conservative theoretical calculations. Potential impacts could occur if releases of flowback water occurred to limited flow aquatic environments.

MP222 Effect of Hydraulic Fracturing Fluid Components on Chironomus riparius Larvae as a Marker for Aquatic Toxicity of Freshwater

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One modern technology that concerns many environmental scientists these days is hydraulic fracturing. Hydraulic fracturing extracts oil and gas from deep underground and has expanded into areas of the United States where production was once considered impractical. Given the increase in hydraulic fracking, there is a risk that chemical components of fracturing fluids could enter fresh waterways and impact aquatic organisms. FracFocus disclosure data lists additives used in fracturing fluid of 39,000 individual oil and gas production wells in the U.S. Additives include sodium chloride as a breaker, friction reducer, scale inhibitor and clay control chemical, and tributyl tetradecyl phosphonium chloride (TTPC) as a biocide to minimize bacterial contamination of hydrocarbons and to reduce bacterial production of corrosive by-products in order to maintain wellbore integrity and prevent breakdown of gellants. Waste water produced from hydraulic fracturing can contain total dissolved solids and Cl exceeding 100,000 mg/L. According to HALLIBURTON company, the concentration of TTPC in fracturing fluid is 0.03% (300 mg/L). In this study, the acute toxicity of TTPC biocide and NaCl were tested using 4th instar Chironomus riparius Results showed that the 48 h LC50 of TTPC was 0.48 mg/L and the LC50 of NaCl was 9368 mg/L. As a part of verifying the mechanism of action of the components, ATP was measured from hemolymph samples of the larvae raised in various concentrations and collected at 6, 12, and 24 h. Results showed that increased levels of TTPC corresponded with decreased levels of ATP. The decrease in ATP levels might be due to loss of cellular and/or mitochondrial membrane integrity.

MP223 Environmental Quality Implications of Unconventional Natural Gas Development: Current Perspectives and Research Needs

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Natural resource extraction (NRE) activities associated with fossil fuels and chemical feedstocks remain a pressing societal need despite the growth of renewable raw material sources. In recent years, few NRE technologies have received as much attention from the scientific, regulatory and public communities as hydraulic fracturing (HF), commonly known as “fracking”. Though HF is increasingly used to increase the efficiency of conventional natural gas development in shales, coal seams, and tightly compacted sands, the potential risks of HF and related natural gas development on public health and the environment require assessment and management. It is clear that interdisciplinary scientific approaches are necessary to define the implications of HF and other related NRE activities to environmental quality, and to develop appropriate environmental management strategies to meet environmental protection goals. Further, the role that environmental fate processes (e.g. biotic and abiotic degradation, bioaccumulation) play in determining both environmental and human exposures warrants additional scrutiny and management consideration. SETAC held timely Focused Topic Meeting in March 2016 to facilitate information exchange on the state of the science on these focused topics and provide a forum for stakeholder engagement. In addition, breakout groups with scientists and engineers from academia, business and government identified research needs in five areas: 1. Environmental fate assessment of fracturing fluids; 2. Chemical hazard assessment of fracturing fluids; 3. Environmental risks to water resources; 4. Environmental risks to land resources; and 5. Environmental risks of produced water for beneficial reuse. This presentation presents context for these research questions necessary to understand the environmental quality implications of unconventional natural gas development.

MP224 PBT Assessment of Hydraulic Fracturing Fluids from Coal Seam Gas Fields in Australia

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The science and fluids used in hydraulic fracturing are constantly evolving, and involve a broad mixture and variety of constituents. Hydraulic fracturing wastes and production water flow containing these constituents have to be disposed or recycled. Coal seam gas (CSG) production in Queensland, Australia, requires a hazard assessment to use and manage these fluids as part of the requirements of the Environmental Authority and the Environmental Impact Statement. The hazard assessment process was conducted to evaluate the constituents used in the hydraulic fracturing fluids that potentially are a concern to human health or the environment based on an assessment of persistence, bioaccumulation, and toxicity (PBT). The PBT assessment provided a framework to assess management requirements, long term environmental risks, and the use of alternative chemicals to produce hydraulic fracturing fluid systems with low toxicity and greater potential for on-site management and reuse. The PBT assessment for the proposed hydraulic fracturing constituents was conducted in accordance with Australian best practice and national or international standards and guidelines. One of the challenges in compiling the necessary PBT data was the proprietary nature of the constituents in the hydraulic fracturing fluids, and the limited experimental data. Therefore, for constituents where available data may not allow a definitive conclusion on the PBT properties, PBT assessment guidance developed by Department of the Environment, Water, Heritage and the Arts, or the PBT assessment guidance for the EU REACH screening criteria, was used to decide whether a substance may potentially fulfill the PBT criteria. Physical-chemical properties, environmental fate and transport, and aquatic and mammalian toxicity data was compiled from routine data used for screening processes (e.g., OECD-SIDS program). The results of a PBT assessment in support of a quantitative risk assessment, and the application to the assessment of two hydraulic fracturing fluid systems to determine which of the two presents a lower overall PBT assessment, will be discussed. The challenges that were encountered because of the proprietary nature of the hydraulic fracturing chemicals are presented, and include confidentiality issues, lack of robust experimental data, and the lack of estimation models.

MP225 Potential Environmental Impacts of Shale Development on Proximate Flora

R. Lupardus, Univ of Northern Colorado / Biology

Chemicals released from active and producing shale wells have the ability to deposit onto surrounding plants, soils or waters, but empirical data are lacking. This deposition might affect species and ecosystem health of proximate flora. We examined potential impacts on Pawnee National Grassland (PNG) vegetation due to shale energy development and release of carcinogenic hydrocarbons referred to as BTEX: Benzene, Toluene, Ethyl Benzene, Xylene. The objective of the research was to quantify the deposition and accumulation of BTEX onto proximate flora. We hypothesized that deposition and accumulation of BTEX onto proximate flora would be greater when wells were pumping and that deposition and accumulation would decrease with time since production. Such knowledge is crucial for science-based development of air pollution control and management strategies. In the spring of 2014 a total of 360 vegetation samples were collected from 5 previously and 15 currently producing shale wells on the PNG. Samples were collected along 50 meter transects in W, NE and SE directions at 20m, 50m and 100m from the production source. Vegetation samples from collection sites were measured for BTEX using gas chromatography and flame ionization detector. Results from the analysis indicate that BTEX were present in a majority of the samples. The mean concentrations (BTEX mg/g veg) for all samples were: Benzene 1.36 E-05, Toluene 2.36 E-06, Ethylbenzene 7.88 E-07, o-Xylene 6.87 E-07 and p-Xylene 8.99 E-06. The highest concentration across all samples was for o-Xylene. Deposition and accumulation of BTEX onto proximate flora was significantly greater, F (9.58) and $p < 0.001$, when wells were pumping, as predicted. Benzene was greater than 30ppm for wells that were pumping up natural gas during the time of sample collection. There was a significant difference between production groups for Benzene F (34.65) and $p < 0.0001$, Toluene F (35.11) and $p < 0.0001$ and o-Xylene F (9.58) and $p < 0.001$. Deposition and accumulation of BTEX onto proximate flora decreased with time since production. These results could have implications for cattle restrictions and safety requirements for, not just cattle, but other wildlife and humans, with daily exposure in close proximity (e.g., within 100m) of a shale production site.

MP226 Framework for an Environmental Effects Monitoring Program for Unconventional Oil and Gas Development in Appalachian Regions of Ohio and West Virginia

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The Utica/Point Pleasant shale play in eastern Ohio and northwestern West Virginia is the focus of the development of high volume horizontal hydraulic fracturing (HVHHF) oil and gas wells at an unprecedented rate. Concerns exist regarding the environmental and human health risks associated with the hydraulic fracturing process, yet data and methods used to frame these arguments, from both environmentalist and industry perspectives, often lack scientific rigor, transparency, and completeness. Assessing the nature of environmental hazards and risks associated with HVHHF processes, chemicals, and waste products in a controlled, scientific manner is limited by ready, consistent access to these procedures and materials from actual drilling facilities. As a result, very few scientific data are available to the public on the environmental risks posed by drilling to surface and subsurface environments. Additionally, the lack of a comprehensive framework to address the multitude of potential effects of HVHHF often leads to the “cherry-picking” of data and lack of a “big picture” interpretation of effects. Only through ongoing access to active, “transparent” drilling sites with organized well development, sampling strategies, and a unified assessment framework can hypothesis-driven research be conducted to inform best practices to reduce environmental and human health risks associated with shale development. To this end, the Ohio State University is developing the Utica Shale Energy and Environmental Laboratory (USEEL). The USEEL represents a unique partnership between industry, academia, and government in the collection of data to assess the environmental and human health risks associated with HVHHF, as well as baseline lithology, chemistry, mineralogy, and microbial biodiversity contributing to geologic history and hydrocarbon development in shale formations. The first step in the development of a comprehensive plan to assess the potential environmental effects of HVHHF is to build a comprehensive framework based upon underlying, large-scale characteristics of the region in which the study will be conducted. This presentation will outline the use of ecoregions, watersheds, hydrologic unit codes (HUC), and the importance of GIS geospatial database tools in setting the stage in Phase I of developing an environmental effects monitoring framework for HVHHF.

Aquatic Toxicology and Ecology – Poster Only – Part 1

MP227 Do Eukaryotic Species Interactions Drive Freshwater Harmful Algal Blooms Dynamics?

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Harmful algal blooms (HABs) constitute an increasingly frequent and severe threat to human health and the environment worldwide. The USEPA's research into how to anticipate and mitigate HABs emphasizes the important role of abiotic factors such as nutrient pollution and temperature. Though informative, these research efforts may be insufficient by themselves. Accounting for the influences of biotic interactions such as predation, disease, and competition on HAB formation/dissipation may improve the power of HAB prediction and mitigation strategies. In the present study, we use a metabarcoding approach to profile changes in the aquatic community of a multipurpose reservoir in southeastern Ohio, USA, that has recently become prone to summertime blooms of toxic cyanobacteria. Our preliminary data indicate a consistent pattern in which HAB formation coincides with conspicuous spikes in the abundance of herbivorous crustacean zooplankton and rapid declines of green algae. Ongoing analyses are intended to elucidate key community dynamics foretelling HAB formation/dissipation. In addition, controlled laboratory experiments will be used to test hypotheses regarding mechanisms driving these dynamics.

MP228 Consequences of soil erosion and cyanobacterial blooms on the feeding behaviour of a native fish Goodea atripinnis (Goodeidae)

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Eutrophication and cyanobacterial blooms are factors associated with environmental degradation and water pollution by mineral nutrients, which could change the zooplankton consumption by the fish. Turbidity caused by both organic (e.g., cyanobacterial blooms) and inorganic (e.g., soil) particles affect the prey visibility. Little is known about the effect of this two kinds of turbidity on feeding behavior of fish, which mostly depend on vision to pursue their prey. Goodea atripinnis is a member of Goodeidae with restricted distribution in Central Mexico. The aim of this study was to determine if turbidity influences the preference and consumption of cladocerans by G. atripinnis. Prey preference experiments were performed under conditions of organic (Microcystis aeruginosa) and inorganic turbidity (clay) at two levels, 60 and 120 NTU. Five cladoceran species were used as prey (Ceriodapnia dubia, Alona glabra, Simocephalus vetulus, Moina macrocopa, Diaphanosoma birgei) and offered at a density of 1 ind. ml^{-1}). The tests were performed using 50 ml of moderately hard water as medium. Two juvenile individuals of the fish were added and allowed to feed on the mixed cladocerans for 30 minutes. At the end of the experiment the fish were removed and the uneaten prey items in the medium were counted. For determining prey preference Manly's α was used. Our tests showed that turbidity modified the prey preference. Compared to controls, fish in turbidity preferred higher number of prey species. This also increased with increasing levels of turbidity. Further, our results showed that high levels of turbidity adversely affected the prey consumption by G. atripinnis.

MP229 Sub lethal concentrations of Cylindrospermopsin causes lipogenesis, cell cycle disruption, and activation of stress responses on hepatocytes

C. Gonzalez, Univ of São Paulo / Clinical Analyses; E. Pinto, Univ of São Paulo / Faculty of Pharmaceutical Sciences; P. Kubiniok, P. Thibault, Universite de Montreal / Inst for research in immunology and cancer

The production of cyanobacterial toxins in Brazilian continental water reservoirs is widely known and a current public health issue. Cylindrospermopsin (CYN) is a hepatotoxin whose excretion was initially reported by Cylindrospermopsis spp., but later found to de produced by many other cyanobacterial genera. Several toxicity mechanisms have been described for CYN on mammalian cell cultures. The most prominent effect is protein synthesis inhibition, although this mechanism is not yet clear. In order to have a deeper understanding of the consequences of CYN exposure on human hepatocytes, we chose a relative quantitative proteomics approach. Briefly, we quantitated the effects of sub lethal doses of CYN on the up regulation and down regulation of proteins from the overall HepG2 proteome. Here we present results from a shotgun proteomics experiment using stable isotope labeling of amino acids in cell culture (SILAC) and nanoscale LC-MS^2 techniques. Previous results from our laboratory using flow cytometry showed that the treatment of HepG2 cells with 1 µM of CYN during 24 hours, did not produce apoptotic nor necrotic effects. Furthermore, the stimulation of HepG2 cells with 5 µM of CYN during 12 hours, did not caused cell death either. Using these CYN concentrations HepG2 cells were treated during several time intervals up to 24 hours. Of all the biological effects produced by CYN on the proteome of HepG2 cells, lipogenesis was the most constant effect. In many of the time points of the experiment, we detected an up regulation of enzymes related to cholesterol biosynthesis, acetyl-CoA biosynthesis, bile acid biosynthesis and Krebs cycle. Moreover, we found evidence of up regulation of proteins related to cell cycle control, for example MCM proteins involved in control of chromosomal replication, and up regulation of transport proteins related to RAN signaling. Notably cell stress-response proteins showed alterations at concentrations previously considered as sub lethal. Examples of up regulation and down regulation of stress response related proteins include processes like glutathione-mediated detoxification, Nrf2 mediated oxidative stress response and positive acute response proteins. Finally, another relevant effect of the toxin throughout the experiment was the up regulation of enzymes involved in the degradation of several amino acids, most of them aromatic AA. A more comprehensive interpretation of these findings will be available at the meeting.

MP230 Stream Susceptibility Profiles in the Appalachian Mountains

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Tennessee's Ecologically At-Risk Streams – Appalachian Mountain (TEARS-AM) is a three-year eco-toxicological study designed to establish the susceptibility of four naturally reproducing eastern brook trout (Salvelinus fontinalis) streams spanning the latitudinal gradient of Tennessee's Appalachian Mountains. By collecting and examining baseline chemical, geological, biological, and ecological data within these streams, TEARS-AM provides the framework required to determine the stream susceptibility profile and establishes long-term monitoring stations for future studies. Year one data revealed all fish tissue was below detection for polychlorinated biphenyls (< 12.5 PPB) across the four sites while mercury and methyl-mercury were above detection (>3.0 PPM) but at different concentrations based on the site. Water quality results showed an average concentration of 1.04 mg/L, 0.31 mg/L, and 0.908 ng/L for total suspended solids, nitrate/nitrite, and total mercury, respectively when data from all four sites were combined. The abiotic and environmental factors affecting these results will be discussed as well as future plans.

MP231 Fish Captured in Canoas River, SC, Brazil, Exhibited Alterated Concentration of Sex Hormones and Gonad Morphological Alterations

C. Soares, Univ Federal de Santa Catarina / Biochemistry Dept; I. Baptista, A. Paiva, Univ Federal de Santa Catarina

The Canoas River is one of the most important in the Santa Catarina state, Brazil. Several studies have been carried out in several sites of this river, especially in the municipalities of Correia Pinto and Ponte Alta, and the results demonstrate that pollutants are being released in its waters, causing physiological and biochemical changes in fish. This study evaluated possible biochemical and physiological changes, especially changes in the levels of sex hormones such as estradiol and testosterone in native fish, Jundiá (Rhamdia quelen) captured in the city of Otacilio Costa, near a paper and pulp mill. The fish were caught with hook and line, and immediately blood samples were taken using heparinized syringes. Liver, gills and the gonads were also removed, which were weighed and then placed in buffered formalin, 10% for histological analysis. Analyses of the concentration of the testosterone and estradiol showed that fish collected at a site downstream of toxic waste discharge had higher testosterone levels than fish caught in a site upstream. Plasma cholesterol concentration was higher than the control group. For every 10 fish caught nine fish were males, in the site affected by the discharge of effluents, in contrast to the control site. Likewise, histological analyzes showed significant alterations in gills, liver and gonads, particularly in females. The observed biological changes can be correlated with physical and chemical changes of the water, particularly the amount of total phenolics. Support: Fapesc/CNPq

MP232 Genetic diversity of a sedentary fish species from a Neotropical stream under anthropogenic interference

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Freshwater fishes have experienced declines in their populations worldwide, as consequence of anthropogenic impacts. Environmental pollution, habitat fragmentation, forest destruction, undesirable agricultural practices are among the main threats to freshwater fish fauna. Whatever the cause, is well established that both loss of species diversity and population declines are usually accompanied by a reduction in genetic diversity. In the Neotropics, the anthropogenic threats to freshwater ecosystems are especially intense and persistent, putting at risk one of the richest and most diverse fish faunas in the world. In our study, based on microsatellite markers analysis, we assessed the genetic diversity of Geophagus brasiliensis, a Neotropical fish that exhibits sedentary habits and parental care, showing low levels of genetic diversity. Samples of G. brasiliensis were collected in three sites (high: H, medium: M and low: L) along a stream of 32 km of extension, located in southern Brazil. In its medium section, this stream receives waters from a small tributary that crosses the urban area, which has been impacted for decades with sewage discharge. Individuals of G. brasiliensis (H: 29; M: 23 and L: 30, n=82) were genotyped in eight microsatellite loci. Measures of genetic diversity varied little among samples. The lowest observed and expected heterozygosity values were obtained for sample from H site (H_O = 0.490 and H_E= 0.578), while the highest value was detected for M (H_E = 0.729). The highest mean of alleles per locus (N_A = 6.000), number of effective alleles (N_E = 4.221) and allelic richness (R_A = 5.939) were obtained for the M sample, while the lowest values for these index were obtained for H sample (N_A = 4.500, N_E = 3.124 and R_A = 4.361). Significant F_{IS} values were obtained in all samples. The sample from site M showed the highest value (0.167) and L showed the lowest value (0.081). We also detected significant heterozygote- excess (bottleneck signal) in M and L samples, in both IAM and TPM models. Thus, despite the neutrality of the markers employed, our findings suggest a genetic bottlenecks signs in M and L samples, precisely the two sites downstream the urban tributary stream. Thus, we cannot discard that anthropic impacts could be acting as a selective force on downstream's populations of G. brasilensis.

MP233 Evaluation, identification, and reduction of sources and causes of sublethal toxicity in the effluent discharge from a former mining site

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A toxicity reduction evaluation (TRE) was completed to identify sources and causes of intermittent sublethal toxicity to Ceriodaphnia dubia (≤ 30% reduction in reproduction) in effluent discharge from a former vanadium mine, and to inform actions to reduce this toxicity. Vanadium was mined at the site from the 1960s until 1986. The TRE combined split-sample toxicity testing among laboratories, spiked effluent testing, toxicity identification evaluation (TIE), and chemical analysis of discharge and contributing water sources (subwatersheds) to evaluate causative factors (e.g., TDS, sulfate, metals) and laboratory variability. Sampling and testing was completed from December 2013 to November 2015 including samples collected during rain events. During this period, insufficient levels of toxicity in the discharge precluded direct identification of chemical causes of toxicity. Toxicity tests on effluent spiked with $CaSO_4$ demonstrated toxicity could not be attributed to SO_4 in the discharge. A toxicity source evaluation showed seep water from a mine spoil area caused sublethal toxicity at concentrations of 5% to 25%. Analysis of flows indicated this seep comprised more than 11% of the effluent up to 50% of the time, and was a likely source of toxicity in the discharge. TIE and chemical evaluation of seep water from the mine spoil area suggested concentrations of manganese (Mn), cobalt (Co), and nickel (Ni), acting alone or together, could account for the observed sublethal toxicity. Additionally, variation among 3 laboratories in the results of split-sample toxicity tests suggested laboratory variability was a factor in evaluating sublethal toxicity to C. dubia, especially at the low levels of sublethal toxicity observed. This information was used to inform site specific reclamation projects. Extensive reclamation focused on covering spoil piles with an impermeable barrier to minimize infiltration of surface water into underlying spoil material and reduce the amount of seepage from the spoil area. Further completion of these activities in the spoil area will focus on minimizing infiltration of surface water into underlying spoil material, reducing seepage, and reducing concentrations of dissolved constituents including Mn, Co, and Ni. Performance monitoring to date indicates continuous improvement in seep management toward the elimination of sublethal toxicity, and discharge biomonitoring will continue as site reclamation proceeds.

MP235 Evaluation of the water quality in dam Tenango, Puebla

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Dams are hydraulic structures that store water for the population benefit, an example of this is the Tenango de las Flores dam on the north of Puebla State, Mexico. It belongs to the Necaxa hydroelectric system and is connected to the Acatlán and Necaxa dams through tunnels. Tenango supplies water to Necaxa for the generation of hydroelectric energy. Corn, coffee and flowers are cultivated in the surrounding areas and there is a protected woods area of pines and oaks, and medium rainforest. At the same time, the dam is used as a tourist attraction and for fishing. The objective of this study was to assess the water quality of the Tenango dam. Five samplings were conducted in a year. Water physicochemical parameters were measured in situ (pH, dissolved oxygen and temperature). Levels of nutrients were analyzed (nitrites, nitrates and phosphorus); as well as metals (cadmium, chromium, copper and lead). Metals were also measured in fish. The results indicated that the physicochemical parameters are within acceptable levels. Nitrites and phosphorus concentrations always exceeded the acceptable limits for urban use and for protection of aquatic life. Lead and chromium exceeded limits on four samplings. Cadmium and copper behaved similarly, exceeding levels permitted by the Mexican legislation for water in two samplings. Metal concentrations in fish tissues were in agreement with water levels. Based on the concentrations of nutrients and metals, it is concluded that the water of the dam is not suitable for urban use, or for protection of aquatic life. These levels of pollution indicate that aquatic life is at risk due to present contradictory uses of water resources in this dam.

MP236 Evaluating the Effects of Livestock Inputs on Fish Populations and Water Quality in the Pine River

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The Pine River, in the Central, Lower Peninsula region of Michigan has a long history of contamination both upstream and downstream of a mill dam located in the town of Alma. Nutrient loading has been measured upstream of the dam due to heavy impacts of livestock facilities and manure application sites along the Pine River and its tributaries. In addition to nutrient loading and decreased levels of dissolved oxygen, excessive concentrations of E. coli bacteria have caused environmental and human health concerns. Additionally, E. coli strains sampled from the Pine River and its tributaries proved to be resistant to multiple antibiotics. E. coli concentrations were quantified in 2015 and were found to exceed safe levels for human contact according to the State Department of Community Health. High bacterial concentrations may affect a large population of anglers that frequently fish in the Pine River at sites where the highest concentrations of E. coli have been reported as well as resident fish populations. The objective of the current study was to evaluate exposure to fish and anglers using both caged and resident fish in the Pine River. Caged juvenile catfish (Ictalurus macrohinus) and bluegill (Lepomis punctatus) were placed at locations upstream of recorded impacts and downstream through locations previously identified as having high concentrations of E. coli. Additionally, resident fish of the Pine River were angled and/or collected via fish shocking at these sites. All fish were identified, evaluated for health, and swabbed to determine concentrations of E. coli. For any E. coli strains identified, antibiotic resistance was determined. At each site where either resident fish were caught or caged fish were placed, general water quality parameters were measured including nutrient and E. coil load. The results of this research may provide the impetus for state officials to address significant and persistent water quality problems sourced in industrial agriculture.

MP237 Effects of anthropogenic activities on aquatic invertebrate communities in an ecologically important conservation area of South Africa

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Temporary wetlands (pans) are some of the most prevalent wetland types worldwide and known as biodiversity hotspots that contribute greatly to regional diversity. Although these aquatic systems are widespread, not only across South Africa but globally, they are not well studied. The Phongolo River Floodplain (PRF) is one of South Africa's largest floodplain systems and is unique to South Africa as it is the only large floodplain that sustains a large number temporary wetlands throughout the dry season. The Ndumo Game Reserve (NGR), located on the PRF, is the only protected area of the floodplain, housing a number of ephemeral and floodplain pans rich in invertebrate biodiversity. Many anthropogenic activities are present in the surrounding area of the NGR, placing a great deal of pressure on the floodplain system. The central aim of this study was to provide baseline information on the biodiversity of aquatic invertebrates found in ephemeral pans across PRF and to determine how this is affected by anthropogenic activities. Water, zooplankton and macroinvertebrate samples were collected from 22 ephemeral pans during February 2014 (wet season). Zooplankton and macroinvertebrates were identified to lowest possible taxon and water analysed for a variety of nutrients. High water temperature and extreme DO concentrations were possible drivers for low aquatic invertebrate biodiversity. Multivariate statistical analyses revealed spatial variation between sites from within the NGR compared to outside the NGR, predominantly driven by aquatic invertebrate biodiversity. A number of pollutant sensitive aquatic invertebrate taxa were found exclusively within the NGR while many pollutant tolerant taxa were prevalent outside the NGR. This suggests that anthropogenic activities are affecting the ephemeral pan biodiversity outside the NGR while the game reserve appears to be protecting biodiversity.

MP238 Changes in Physical Factors following Low-Level Disturbance in a West Virginia Watershed

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Freshwater ecosystems are known to be impaired by disturbances within their watersheds. These disturbances may result from multiple sources ranging from resource extraction to development (e.g. urban, residential, industrial, and commercial). Even low level disturbances have been shown to result in impairment to stream biota within the watershed in landscape-scale evaluations. However, when very low levels of disturbance occur, the mechanisms, which result in impairment to the biological integrity of the freshwater ecosystem, are not well characterized. The objective of this study was to examine the immediate effects of an anthropogenic disturbance of less than 1% of the watershed area on the aquatic ecosystem. Specific parameters presented in this discussion will be changes to the physical factors within the watershed including light intensity, canopy cover, temperature, relative humidity, and evaporation. Baseline conditions were established during a 6-week pre-disturbance period for comparisons of pre- and post-disturbance conditions. A low level disturbance was created by timbering a 0.24 acre area in the 89 acre watershed. Comparisons between pre-disturbance and post-disturbance conditions were evaluated using one-way analysis of variance or Kruskal-Wallis procedures where appropriate. No significant differences were seen in light intensity or canopy cover before and after the disturbance at three monitoring sites located in the upper, middle and lower reaches of the watershed, indicating that the disturbance was not close enough to the stream to affect these physical factors. Evaporation rates were significantly higher ($F=7.03$; $p< 0.05$) and humidity was significantly reduced ($X^2=33.04$; $p< 0.01$) after the disturbance with the greatest effect noted at the monitoring site located in closest proximity to the disturbance. Evaluating the effects of the disturbance on temperature and humidity are complicated due to seasonal variability.

MP239 Assessment of DNA damage in freshwater mussels from the Hamilton Harbour watershed

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A variety of chemicals can induce damage to the DNA of animals, which, if not repaired, can lead to a cascade of biological effects at any level of biological organization, from cellular to community and population level. First developed in the 1980s, the single cell gel electrophoresis assay – or Comet assay – is used to visualize and quantify cellular DNA damage. In recent years, a number of studies have used this approach to evaluate DNA damage from exposure to specific chemicals (e.g., PAHs), as well as from complex mixtures of contaminants, such as industrial or municipal effluents. The objective of the present study was to validate the Comet assay in wild freshwater mussels and to quantify the DNA damage in mussels from the Hamilton Harbour watershed in comparison with that in mussels from reference sites in southern Ontario. Giant Floater mussels (Pyganodon grandis) (91 ± 18 mm, n = 68) were collected from three sites: Cootes Paradise; a small stream directly under highway 401 near Campbellville; and in a small agricultural stream in Embro, Ontario. The mussels were brought to the laboratory and held overnight, a small quantity of hemolymph (blood) was collected the next morning, and the mussels were returned to their habitat of origin within 24 hours. The density and viability of hemocytes (blood cells) were quantified by flow cytometry, and the presence and relative quantity of DNA strand breaks were quantified using the Comet assay. The catch per unit effort was lower in Cootes Paradise compared to Campbellville and Embro. Cell density was significantly elevated in the hemolymph of Giant Floater mussels collected in Campbellville and Cootes Paradise, compared to the hemolymph of those collected in Embro. The hemocytes of mussels from Cootes Paradise had significantly greater DNA damage than those of the mussels from Campbellville and Embro, suggesting exposure to genotoxic compounds. As it has been linked to reduced growth, abnormal development and reduced survival, DNA damage, if not repaired, could have repercussions at the individual and community level. Validation of the Comet assay in other aquatic species, and follow-up studies in Hamilton Harbour AOC are under consideration.

MP240 Hydrocynus viittatus's potential as bio-indicator of riverine health: A multiple lines of evidence and multivariate statistics approach

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There is a need for sensitive bio-monitoring and indicator tools in toxicant impact assessment to show the effect of toxicants on fish health in polluted aquatic ecosystems. Histopathological and biomarker assessments of fish tissue are a bio-monitoring tool and allows for early warning signs of disease and detection of long term injury in cells, tissues or organs. The aims of this research was firstly to determine and compare the health status of the tigerfish (Hydrocynus vittatus) from two lowland rivers in Kruger National Park (KNP) that currently have different anthropogenic impacts, secondly to attempt to explain the histological and biochemical changes observed, through the application of a suite of multivariate statistics to relate the changes to biotic levels of selected metals and organochlorine pesticides (OCPs) and thirdly to determine the suitability of H. vittatus as a bio-indicator of riverine health. Tigerfish were caught using rod and reel from the Olifants River (n=37) and the Luvuvhu River (n=34) between 2009 and 2011. Even though fish were considered to be healthy, the general fish health in both rivers improved over time, corresponding to an overall decrease in river pollution, especially in terms of metals in muscle tissue. The incorporation of multiple lines of evidence in tigerfish, including histopathological changes and cellular, organ and whole organism indices, proved to be a valuable tool in using a bio-indicator approach toward river monitoring. These changes serve as an early warning system to more serious health concerns arising if the pollution in the rivers of the KNP is not dealt with. The use of a suite of uni- and multivariate statistics proved helpful in determining the links between fish health and river contamination and further proved to be a valuable tool in assessing spatial and temporal differences in river pollution and the effects thereof on the selected bioindicator.

MP241 Zebra Mussels as Bioindicators of Habitat Quality of the Great Lakes USA

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Bivalve mollusks are valuable bioindicator species due to their sessile lifestyle and filter-feeding capabilities, and their tendency to accumulate contaminants in their tissues. Therefore, bivalves can provide spatial and temporal insights for identifying habitat stress. Freshwater systems can experience persistent anthropogenic inputs (industrial/agricultural runoff, land development, etc.), which can cause long-term impacts on biota and ecosystem health. NOAA's Mussel Watch program has been used to monitor contaminants in aquatic systems and recently has been expanded to include the US Great Lakes as part of the Great Lakes Restoration Initiative. Caging studies were conducted with zebra mussels (Dreissena polymorpha) to evaluate the potential toxicity and bioavailability of contaminants from Great Lakes sites in Lake Michigan and Lake Erie in collaboration with NOAA. NOAA staff conducted caging studies during the summer of 2012 at sites dominated by urban inputs and high levels of PCBs and PAHs (Manistique River, Menominee River, Green Bay, Sheboygan River, Ashtabula River, and Milwaukee Bay); and during the summer of 2015 at the Maumee River, dominated by agricultural runoff and elevated pesticides (such as chlorpyrifos). Harvested mussels were sent to UNC-Charlotte for cellular biomarker analyses (lipid peroxidation and total glutathione for all sites and acetylcholinesterase (ACHE) for the 2015 samples); tissue samples were processed for contaminants by NOAA. There were highly significant correlations between the tissue concentrations of PCBs and PAHs and glutathione levels. Changes in glutathione levels and lipid peroxidation, indicated significant oxidative stress. Increased ACHE levels were also observed in mussels from the Maumee River. These studies indicate that while zebra mussels are typically branded as an invasive species, they can also be a valuable bioindicator species to assess habitat quality and ecosystem health in the Great Lakes and other freshwater locales.

MP242 Navajo Generating Station ERAs: A Successful Communication Strategy that Facilitated the ERA Process Associated with the NGS EIS

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Navajo Generating Station (NGS) is a coal-fired electric power generating station located in northern Arizona on land leased from the Navajo Nation. Ecological risk assessments (ERA) were conducted over five discrete areas to evaluate environmental conditions in the vicinity of the NGS to inform the environmental impact statement conducted for compliance with the National Environmental Policy Act and the Endangered Species Act Section 7 Consultation for the federal decisions to authorize NGS operations beyond 2019. A key challenge for the project was to implement technical ERA approaches that allowed for input from multiple stakeholders with widely varying technical knowledge levels, goals and objectives. Stakeholders included the Bureau of Reclamation, the Office of Surface Mining Reclamation and Enforcement, the US Fish and Wildlife Service, the Bureau of Indian Affairs, the US Environmental Protection Agency, the Navajo Nation, Hopi Tribe, and National Parks Service. This presentation focuses on the communication strategy implemented for the project that simplified the understanding of technical concepts, encouraged opportunities for consensus building, endorsed real-time contributions to the ERA process, and facilitated the development of ERA documentation appropriately suited for the needs of the EIS.

MP243 Tracing Deepwater Horizon Oil into Coastal in situ Bacterial Communities

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One year after the Deepwater Horizon oil spill, samples were collected from Barataria Bay, LA (heavily-impacted), Pensacola Bay, FL (moderately-impacted), and Apalachicola Bay, FL (non-impacted) to evaluate spill effects on indigenous bacterial communities. Natural Delta ^{14}C and del ^{13}C abundances were utilized to trace in situ bacterial hydrocarbon remineralization to dissolved inorganic carbon (DIC). Barataria Bay DIC samples were significantly deplete in Delta ^{14}C (-109.98 per mil to +12.48 per mil) relative to that for Apalachicola Bay (+36.31 per mil) indicating hydrocarbon remineralization at the Barataria Bay site. Using dual isotope three endmember mixing models we estimate that between 1 to 12% of the respired DIC at Barataria Bay can be attributed to hydrocarbon remineralization. Bacterial abundances were an order of magnitude higher in Barataria Bay when compared to the Apalachicola Bay which correlated with higher DOC concentrations at the Barataria Bay site. Clone libraries indicate distinct differences in bacterial community structure between sites due to increases in the presence of known oil degrading bacteria in Barataria Bay and Pensacola Bay. Collectively, these results demonstrate that Barataria Bay bacterial communities did utilize oil and show that such a combined biogeochemical and molecular approach can be a powerful tool for evaluating oil intrusion into the marine food web.

MP244 Toxicity Laboratory Intercalibration for Stormwater in Southern California

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Because of the expansive urbanization of watersheds in southern California, the monitoring of stormwater has become increasingly important. Nearly a million dollars is spent each year monitoring stormwater toxicity. This work is funded by multiple agencies, conducted by many different laboratories, and uses several test organisms. A group of the agencies, the Southern California Stormwater Monitoring Coalition, has a goal of combining all of the data sets that have been generated to make spatial and temporal comparisons. This is challenging because while the laboratories are using standardized test methods, there are options and room for interpretations in the protocols that allow some variation in procedures. As a result, an intercalibration exercise was conducted to assess the comparability of results between laboratories. A total of nine laboratories tested split samples of dilution water, copper spiked dilution water, simulated runoff and a duplicate of one of these matrices. In the first round, tests were conducted using survival and reproduction of water flea Ceriodaphnia dubia, 96 hr. survival of the freshwater amphipod Hyalella azteca, 48 hr larval development of the salt water mussel Mytilus galloprovincialis, and 72 hr larval development of the sea urchin Strongylocentrotus purpuratus. Each laboratory was allowed to use their normal protocols. The round one results found good comparability for the two marine tests, but relatively poor comparability for amphipod survival and water flea reproduction. A survey of the laboratories indicated several differences in protocols for the two freshwater tests. A second round of testing for just the freshwater tests occurred with more standardization of procedures. Comparability of the amphipod test was greatly improved, but the water flea reproduction endpoint remained relatively poor. For both rounds, the most comparable results were achieved on the simulated runoff samples indicating a possible matrix issue with dilution water samples between laboratories.

MP245 Impact of Metropolitan Stormwater Runoff on Fathead Minnows (Pimephales promelas)

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Aquatic toxicology has focused on the testing of single compounds and easily traced point source pollution, such as that found in mining, agriculture, and pharmaceutical industry. The focus of this study was to identify how a complex mixture of compounds, found in a major metropolitan stormwater system, would affect body condition, feeding efficiency and survival of fathead minnows (Pimephales promelas). Stormwater runoff was collected during snow melt, two spring and one summer rain event from nine locations in St. Paul and Minneapolis, Minnesota. Twenty-one-day static renewal exposures were conducted for the snow melt and first spring rain events using larval fathead minnows, followed by analysis of competitive feeding assay, body length, and predator escape performance. Results from the snow melt fathead minnow exposure indicate differences in feeding efficiency (ANOVA with Holm-Sidak's post-test, p=0.04,) between stormwater collection sites. Spring rain event exposed fathead minnows display differences in mean body length of 13% and 17%, respectively, shorter than control larvae, in minnows exposed to runoff collected from two inflows to stormwater retention ponds (ANOVA with Holm-Sidak's post-test, p=0.0011).The result of the fathead minnow exposures suggest adverse impacts of stormwater exposure on feeding efficiency and body condition and more study should be devoted to this underexplored source of aquatic contaminants.

MP246 Presence of fecal indicator bacteria in sand by distance and depth at a freshwater recreational beach

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Escherichia coli (E. coli) is a fecal indicator bacteria (FIB) the Environmental Protection Agency and other regulatory agencies use as a surrogate to assess potential water quality impairment from fecal pathogens. Typically, E. coli is assessed in water with little emphasis on other environmental matrices, such as sediment or sand, which can serve as secondary habitats for fecal bacteria once they reach aquatic systems from storm water runoff or other sources. The objectives of this study were to investigate the presence of E. coli in sand at depth (0-10, 10-20, and 20-30 cm) and distance from shoreline (0, 3, 6, 9, 12 m) at a freshwater, inland recreational beach. Samples were collected at 25 transect points from dry beach sand (onshore) and wet sand (offshore) and analyzed for E. coli using the IDEXX culture method. The highest concentrations of E. coli (~8,000 cfu/100g sand) were found in the 0-10cm sections and closest to shore. Findings of FIB in inland beach sand could prompt regulators to create criteria for protection of recreators from potential pathogens in beach sand.

MP247 Antibody Responses to Bacterial Pathogens in Loggerhead, Caretta caretta, and Kemp's Ridley, Lepidochelys kempii, Sea Turtles in the S.E. USA

M.L. Rodgers, C.D. Rice, Clemson Univ / Biological Sciences

Sea turtles are subjected to multiple environmental stressors, including, but not limited to, habitat encroachment, thermal stress, environmental contaminants, plastics, and various pathogens. How these stressors interact to impact the health and immunological fitness of sea turtles is not well understood. One obstacle in better understanding sea turtle immune functions and responses to pathogens is the lack of critical reagents (namely antibody reagents) that recognize their immunoglobulins (Igs). Turtle IgY is analogous to IgG in higher vertebrates, and is similar in function to IgY of birds. Importantly, IgY is the main class of Igs responsible for long term immunological memory in reptiles, and can serve as a marker of exposure and magnitude of response to pathogens, and especially responses to specific antigens derived from pathogens. This study focused on two sea turtle species: loggerhead (Caretta caretta) and Kemp's ridley (Lepidochelys kempii). Several methods to purify IgY were employed, but the most successful technique used Protein A/G columns under low salt concentrations. The final purified product consisted of 65 kDa heavy chains, 23 kDa light chains, and a 40 kDa F(ab)2 fragment, which is similar to bird IgY, though this purification technique does not work in chickens. Purified IgY was used to immunize mice for antibody production. Using these antibody reagents, serum samples from 60 loggerheads and 30 Kemp's ridleys were probed by ELISA and western blots for both total and bacteria-specific IgY antibody responses. Of the nine species of marine bacteria investigated as targets for measuring antibody responses, IgY responses were highest against, E. coli, V. parahaemolyticus, M. marinum, E. rhusiopathiae, and S. agalactiae. Loggerhead turtles recognized more protein bands from bacterial lysates than did Kemp's ridley turtles. Some correlations were found between total serum protein levels and serum titers against bacteria, and between straight carapace length and serum titers. Other measurements taken from these individual turtles, including blood packed cell volume, glucose, and total protein, are similar to measurements seen in other wild, healthy turtles of the same species from the Chesapeake Bay. These data offer a baseline of information for future studies that continue to monitor the health of these two species in their dynamic environment.

MP248 Exploring Different Service Loss Estimation Approaches for Assessing Sediment Injury in Natural Resource Damage Assessment

M.W. Kierski, Exponent / Ecological and Biological Services Practice; P.N. Booth, Ramboll Environ / Ecological Sciences; M. Edwards, Exponent / Environmental and Human Health Consulting; J. Ma, Exponent

Natural Resources Damages Assessments (NRDAs) for injury to sediment typically rely on the use of Habitat Equivalency Analysis (HEA) as a means of determining the scope of compensatory mitigation necessary to offset lost services due to a release. HEA, in turn, is a tool for estimating the spatial dimension of service loss over time and expresses service losses and gains in units of service-acres-years (SAYs) defined as the level of ecological services provided by one acre of sediment habitat over a one year period under baseline conditions. Application of HEA involves two fundamental steps: 1) development of quantitative relationships between concentrations of one or more constituents in sediment and the inferred ecological service loss, and 2) use of geographical information systems (GIS) to conduct spatial analysis of sediment chemistry to determine service losses by unit area (SAYs). The outcome of any HEA analysis is highly sensitive to the assumptions inherent in each of these steps. This presentation focuses on Step 1 of the HEA and compares and contrasts a variety of different approaches for estimating service loss relationships using a real-world data set for a former Tar plant where data were collected (e.g., polycyclic aromatic hydrocarbon (PAH) sediment concentrations, sediment toxicity bioassays, and benthic invertebrate community data) to evaluate the potential service loss associated with the benthic invertebrate community. The data requirements needed for each approach are discussed, along with the strengths and weaknesses of the different approaches, and the effect on the results of the HEA.

MP249 Lethal and sub-lethal effects of elevated CO2 concentrations on marine organisms

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Concern about leakage of carbon dioxide (CO_2) from deep-sea storage in geological reservoirs is increasing because of its possible adverse effects on marine organisms locally or at nearby coastal areas both in sediment and water column. In the present study, we examined how elevated CO_2 affects various intertidal epibenthic (benthic copepod), intertidal endobenthic (Manila clam and Venus clam), sub-tidal benthic (brittle starfish), and free-living (marine medaka) organisms in areas expected to be impacted by leakage. Acute lethal and sub-lethal effects were detected in the adult stage of all test organisms exposed to varying concentrations of CO_2, due to the associated decline in pH (8.3 to 5.2) during 96-h exposure. However, intertidal organisms (such as benthic copepods and clams) showed remarkable resistance to elevated CO_2, with the venus clam being the most tolerant (LpH50 = 5.45). Subtidal species (such as brittle starfish [LpH50 = 6.16] and marine medaka [LpH50 = 5.91]) were more sensitive to elevated CO_2 compared to intertidal species, possibly because they have fewer defensive capabilities. Of note, the exposure duration might regulate the degree of acute sub-lethal effects, as evidenced by the venus clam, which showed a time-dependent effect to elevated CO_2. Finally, Copper was chosen as a model toxic element to find out the synergistic or antagonistic effects between ocean acidification and metal pollution. Combination of CO_2 and Cu exposure enhance the adverse effects to organisms, generally supporting a synergistic effect scenario. Overall, the significant variation in the degree to which CO_2 adversely affected organisms (viz., working range and strength) was clearly observed, supporting the general concept of species-dependent effects of elevated CO_2 Key words: Carbon dioxide, CO_2 Capture and Storage (CCS), pH, Intertidal organism, Subtidal organism.

MP250 Does parent exposure to municipal wastewater effluent exposure influence progeny health or response to endocrine disrupting compounds?

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Over 30 fish species have been reported to show an intersex condition (ova-testes) after exposure to municipal wastewater effluent (MWWE), suggesting that the feminization of male fish is a widespread phenomenon. Previous studies in the Grand River watershed have shown that male rainbow darter (Etheostoma caeruleum) collected near the Kitchener MWWE outfall are feminized. While it is well recognized that MWWE has direct impacts on the sexual development and performance of the F0 generation, little is known about the consequences of MWWE exposure on subsequent generations of fish. The purpose of this study was to determine whether parent exposure history influenced the health, sexual development or response to endocrine disrupting compounds. Progeny of rainbow darter collected from rural (reference) and MWWE exposed sites were exposed to either de-chlorinated tap water (control) or 10% MWWE from 21 to 100 days post hatch (dph). Endpoints assessed included survival, growth, sexual development, and liver transcriptome. While survival from 21 to 100 dph was not effect by either parentage or exposure group, condition was found to be impacted by both variables. Parent exposure resulted in progeny with reduced condition. Similarly, exposed progeny of parents from the R site had lower condition than non-exposed progeny. Exposure of progeny to MWWE resulted in an increase in intersex incidence. Parental exposure experience increased the likelihood of developing intersex condition in both exposed, and non-exposed groups of progeny. When transcriptomes were compare using a principal component analysis, it was found that progeny of parents that had been exposed, but reared in clean water grouped with MWWE exposed progeny (of both parent groups). These findings suggest that there are transgenerational effects of MWWE exposure.

MP251 The Utilization of Copper Water Effect Ratio Studies for Industrial and Municipal Discharges: A Review of Case Studies

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The development of site-specific water quality criteria for individual metals through the performance of water effect ratio (WER) testing has successfully enabled permittees to modify National Pollutant Discharge Elimination System (NPDES) discharge permit single metal permit limits. This testing concept is based on the U.S. Environmental Protection Agency (EPA's) recognition that the metals can be substantially less toxic in an effluent/receiving water matrix than in clean laboratory water, on which the single metal permit limits are often based. Acute toxicity testing conducted concurrently in site water and laboratory water with the toxicant of concern is used to generate a ratio of toxicity from the test results. One metal in particular that has commonly been evaluated through these studies is copper. The toxicity of copper is dependent on site-specific factors such as pH, hardness, total suspended solids and dissolved organic carbon. Copper WER studies conducted by EA have been utilized by industrial and municipal dischargers to obtain site specific water quality criteria. These studies have encompassed a wide range of hardness values (12-200 mg/L) and have yielded water effect ratios ranging from 2.0 to >56.0. This presentation reviews the data and factors affecting the side by side testing results.

MP252 Sewer Effluent in Our Streams : Can aquatic communities survive?

B. Lognion, Philander Smith College; L.M. Jackson, Philander Smith College / Biology

Domestic sewage effluents represent one of the most common causes of degradation of water quality in stream ecosystems. The effects are especially relevant in ecosystems where water is scarce. There is a great need for considering biotic integrity in assessments of aquatic ecosystems, including taxa composition, diversity, and functional organization of living organisms. Using this approach will provide a broader understanding of the processes going on in altered streams. However, it is not clear what patterns should be expected below sewer treatment plant (STP) effluents. Previous studies reported changes in taxa composition that implied a decrease in taxa richness and an increase in dominance because sensitive taxa were eliminated and resistant taxa were enhanced. Several studies found a decrease in total density of macroinvertebrates with increasing nutrient concentrations, while others reported no changes. Few studies considered functional organization in their assessments and their results are not consistent. Even less is known about the effects of point sources on standing crops of benthic organic matter (BOM) although it is a source of food and influences structure and function of streams. The objectives of this study are to examine the response of the macroinvertebrate community to a STP input in a rural and an urban stream during summer when the dilution capacity of streams is lowest and discharge of point sources has higher adverse effects. Effects of the point source on the structure and functional organization of the benthic macroinvertebrate community will be examined. Also, determination if the self-purifying capacity of the stream results in differences between the community located few meters below the STP input and the community located 500 m downstream. Results will be presented at the meeting.

MP253 Seasonal Variation of Oxidative Stress and Histopathological Alterations in Carassius auratus Affected by Effluent from a Sewage Treatment Plant

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The aim of this study was to identify the influence of effluent discharge from a sewage treatment plant by evaluating oxidative stress and histopathological alterations in crucian carp Carassius auratus collected from upstream, mixing zone and downstream of the effluent discharging point in summer and winter. Sampling was conducted in July 2015 and February 2016. Lipid peroxidation (LPO), catalase (CAT) activity, glutathione S-transferases (GST) activity in gills, liver and kidneys of C. auratus were analyzed. In both seasons, CAT activity of C. auratus collected from the mixing zone and downstream site was significantly higher than that of fish from the upstream site. Histological alterations were also observed in C. auratus and the changes were analyzed semi-quantitatively using mean assessment value (MAV) and degree of tissue change (DTC). The results indicate that the order of magnitude of changes were liver > kidney > gills in both seasons, and the values were higher in samples collected in winter than those collected in summer. In addition, the highest DTC was found in C. auratus collected from the mixing zone followed by downstream site. These findings indicate that fish collected from mixing zone are most affected by effluent discharge and the influence shows seasonal variation.

MP255 Hepatic transcriptome in rainbow darter (Etheostoma caeruleum) exposed to municipal wastewater over time in the central Grand River, Ontario, Canada

P. Marjan, Univ of Waterloo / Biology; M.R. Servos, Univ of Waterloo / Dept of Biology; D. MacLatchy, Wilfrid Laurier Univ / Biology; C.J. Martyniuk, Univ of Florida / Physiological Sciences

There is considerable evidence that contaminants of emerging concern elicit sub-lethal effects at the molecular level in fish residing in urbanized areas. In the present study, a rainbow darter (Etheostoma caeruleum) (RBD) microarray was used to assess hepatic gene expression in fish collected from the Grand River (Ontario, Canada) that had been exposed downstream of the Waterloo and Kitchener municipal wastewater treatment plants (MWWTPs) over four fall seasons from 2011 to 2014. The first objective aimed to determine if transcriptomic responses in male RBDs collected downstream of the two MWWTPs would differ significantly from transcriptomic responses in individuals from a reference site. The second objective was to determine if system upgrades of the Kitchener MWWTP in 2012 resulted in alterations of the RBDs transcriptome indicative of diminished contaminant exposure and system recovery. Temporal responses of differently expressed probes (DEPs) in exposed RBDs differed from those responses in non-exposed RBDs. The magnitude of response in the liver of RBDs exposed downstream of the Waterloo MWWTP was significant in range, and responses varied from 767 to 3,867 DEPs. The range of these responses in RBDs exposed to the Kitchener MWWTP was constricted to 904 to 1,223 DEPs. Gene Set Enrichment Analysis in RBDs from both sites revealed a gradual decrease in the number of impacted gene ontologies over time. Gene network analysis also showed that molecular responses grouped into diverse themes and this was dependent upon the year. There was a noticeable shift in the types of cell pathways affected over time, and the dominant cell pathways affected in the liver previous to upgrades were related to modifications of genetic and cell division (e.g. DNA metabolism, DNA replication checkpoint, genetic stability, genomic instability, autolysis, cell aging) whereas, following the upgrades, cell pathways related to the immune system, reproduction and biochemical processes were preferentially affected. These complex transcriptomic responses in exposed RBDs over time suggest that seasonal factors may play a greater role than the effluent in determining molecular responses. However, it is pointed out that responses are likely the result of both anthropogenic sources and environmental factors, and statistical methods are needed to distinguish the relative contribution of each.

MP256 Chronic Exposure of the Least Killifish (Heterandria formosa) and Mosquitofish (Gambusia affinis) to Treated Sewer Effluent: Can they withstand?

D.T. Bryant, Philander Smith College; L.M. Jackson, Philander Smith College / Biology

The presence of sewer treatment plant (STP) effluents in the aquatic environment has gained major worldwide attention because of the possibility that these effluents may have negative effects the reproductive health of wildlife populations and humans. Concern has been raised that exposure to these effluents can disrupt the endocrine functions of fish exposed to effluent-receiving waters. There is a need for a better understanding of the relative importance of endocrine disruption in relation to sewer effluent toxicity. To investigate impacts of STP effluents on an aquatic environment, a full life cycle in vivo study will be conducted to examine the potential effects of STP effluents in the least killifish (Heterandria formosa) and western mosquitofish (Gambusia affinis). We will test the effects of environmentally relevant concentrations of treated water effluent. Newborn fishes will be exposed to 0, 10, 25, 50, or 75% effluent (with dechlorinated tap water as diluent). Final effluent will be passed through a 100-µm filter before it is supplied to the tanks holding the fish. Solutions will be renewed every 48 hours in a static renewal system. Effects of STP effluent on length, weight, sex ratio, survival rate, time to sexual maturity, vitellogenin induction, population abundance, population dynamics, and incidences of intersex, feminization of males, and masculinization of females in the developing fishes will be examined. Results will be analyzed and presented at the meeting.

MP257 Assessing Load Reduction and Biological Recovery After Wastewater Treatment Upgrades in an Effluent-Dominated Aquatic Ecosystem

H.I. Schoenfuss, St. Cloud State Univ / Aquatic Toxicology Laboratory; D. Martinovic-Weigelt, Univ of St. Thomas; P. Edmiston, The College of Wooster / Chemistry; T. Minarik, MWRDGC / Associate Aquatic Biologist

Many urban aquatic systems are becoming effluent dominated, resulting in the presence of contaminants of emerging concern and subsequent adverse effects on aquatic wildlife. Despite these dramatic alterations, effluent dominated urban systems support many ecosystem services and are used by the nearby human population for recreation. The Metropolitan Water Reclamation District of Greater Chicago is upgrading two billion liter/day wastewater treatment plants to disinfection (UV; chlorination/de-chlorination). The receiving aquatic ecosystem adjacent to these two wastewater treatment plants has been the focus of intense biological and chemical study for the past seven years and provides a unique opportunity to assess two divergent treatment technologies (UV disinfection vs. chlorination/de-chlorination) and to examine how adverse biological effects in exposed fish may be mitigated through effluent disinfection. We exposed male fathead minnows in on-site flow-through exposure systems four times prior to the treatment upgrades and twice since to examine these questions. In addition, we conducted extensive analytical chemistry on effluent samples and employed in vitro assays to examine overall biological activity of effluents prior and following disinfection treatment.

MP258 Biological Effects of Septic Seepage on the Larval Fathead Minnow, Pimephales promelas

L. Warren, St Cloud State / Aquatic Toxicology Laboratory; H.I. Schoenfuss, St. Cloud State Univ / Aquatic Toxicology Laboratory; M. Guyader, C.P. Higgins, Colorado School of Mines / Dept of Civil Environmental Engineering; R. Kiesling, USGS

The potential of On-site Wastewater Treatment Systems (OWTSs) being a non-point source of contaminants into lake systems is a growing concern. Since many lakes are down gradient of OWTSs, the septic seepage easily contacts surrounding groundwater and enters the shallow waters though the hydrological process. Five study lakes were established that included two septic-influenced sites and two reference sites each. To explore the effects of these contaminants on the larval fathead minnow, Pimephales promelas, a 21-day static renewal exposure was completed using pore water collected from septic influenced and reference sites in each study lake. Following the 21-day exposures, larvae underwent behavioral testing that included the analysis of predator avoidance as well as feeding performance. Results indicate declined survival and latency in the septic and positive control treatments. Concurrent pore water analysis will be used to build synthetic mixtures of different septic systems for future laboratory exposures. Exposures like these will help explore the effects of OWTSs on lake ecosystems.

MP259 Genotoxicity and histological damage by anionic surfactant in Zebrafish

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The anionic surfactant dioctyl sodium sulfosuccinate (DOSS) is one of the main active compounds of some oil dispersants, it is also found in laxatives, cosmetics, and detergents. The toxic effects of this surfactant is still under investigation. The exposition to genotoxic compounds can promote the formation of micronucleus (MN) in erythrocytes, a malformation related with genetic damage and can also lead to histological alterations in different tissues. We evaluate the effect of DOSS exposure on adult Zebrafish analyzing its genotoxic potential using a micronucleus test besides histological alterations assessment by Giemsa and Hematoxylin-Eosin staining respectively. A static bioassay was performed for 96 h in glass aquariums containing different concentrations of DOSS: 0, 10, 50 and 80 mg/L (two replicates/treatment). Twenty adult zebrafish were randomly allocated in each aquarium and four fish from each treatment were sampled at random every 24 h. We collected peripheral blood and main organs. Our results suggest a negative effect of DOSS exposure on Zebrafish, with micronucleous formation in erythrocytes detected at 50 mg/L of DOSS at 72 h, and also we found hypertrophy and hyperplasia in gill. The biological implication of this finding is relevant because DOSS is classified as GRASS.

MP260 Effects of perfluorinated compounds on mitochondrial functions in zebrafish liver cells

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Perfluoroalkyl acids (PFAAs) have been widely used in various industrial and consumer applications as synthetic surfactants. Due to their persistence in the environment and biota, it is necessary to identify the adverse effects of these compounds. Many studies have reported toxicological effects of PFAAs on liver, nervous and endocrine systems, and fetal development. Damages in mitochondria and mitochondria-mediated oxidative stress are suspected to cause this wide range of toxicological effects by disrupting cell homeostasis. In this study, we exposed zebrafish liver cells (ZFLs) to perfluorooctane sulfonate (PFOS), perfluorooctanoic acid (PFOA), and perflurononanoic acid (PFNA) to investigate potential mechanism of adverse effects on mitochondrial function. ZFLs were exposed tofour different concentrations (0.05, 0.5, 5, and 50 μM) of PFOS, PFOA, and PFNA. Following 72 h exposures, transcription of genes that make up the electron transport chain (cytb, atp5a1, coxI, and coxIV) and respond to oxidative stress (p53, gstπ, hmox1, and sod2) were analyzed using quantitative real-time PCR. There were no changes in the gene expressions of ZFLs to PFOS or PFOA. However, PFNA-exposed groups showed altered gene expressions in all genes tested except gstπ. Mitochondrial genes cytb and coxI, and oxidative stress response genes p53 and hmox1 were significantly upregulated at 5 or 50 μM ($p< 0.01$). Also, upregulation of sod2 at 50 μM ($p< 0.05$) and of coxIV and atp5a1 at 5 or 50 μM ($p< 0.05$ and $p< 0.01$, respectively) were observed. These initial results draw attention to potential effects of PFNA on various mitochondrial functions. Assessment of damages in the mitochondria membrane and mitochondrial DNA are under way. This work was supported by NRF 900-20150033 (Republic of Korea).

MP261 Bioavailability and Bioconcentration Potential of Perfluoroalkyl-phosphinic and -phosphonic Acids in Zebrafish (Danio rerio)

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Currently, information regarding bioavailability and bioconcentration potential of perfluoroalkyl phosphinic acids (PFPiAs) in aquatic organisms does not exist. The main objective of the present study was to assess uptake and elimination kinetics of PFPiAs in zebrafish (Danio rerio) following aqueous exposure. The results showed that PFPiA exposure can result in very high steady-state bioconentration factors (BCF_{ss}), compared to perfluorocarboxylates and perfluorosulfonates.C6/C10 PFPiA exhibited the highest BCF_{ss}, ranging between 10^7 and 10^{10}, orders of magnitude higher than those for long-chain perfluorocarboxylates. Strong positive relationships were observed between BCF_{ss} versus the membrane-water distribution coefficient (D_{mw}) and the protein-water partition coefficient (K_{pw}) of the studied perfluoroalkyl substances. However, BCF_{ss} exhibited a substantial drop for the very hydrophobic PFPiAs (C8/C10 and C6/C12 PFPiAs). The reduced BCF_{ss} of these long-chain PFPiAs (perfluoroalkyl chain length = 18;$D_{mw} = 10^9$) is likely the result of reduced bioavailability due to interaction with solute molecules/organic matter present in the water phase and/or reduced gill membrane permeability. While PFPiAs can be metabolized to perfluoroalkyl phosphonic acids, the metabolic transformation rate seems insufficient to counteract the high degree of uptake across gill membranes. These findings help to better understand exposure pathways and bioaccumulation behavior of these important perfluorinated acids in aquatic systems.

MP262 Effects of Exposure to Detergents on Freshwater Organisms

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The toxicity of detergents on aquatic organisms has been evaluated since the 60s, but has only been determined the toxic effects of surfactants and there are few studies of the effects of commercial products. Because in our country are scarce studies with detergents, and wastewater from Mexico city have a considerable contribution of of these products, in this study an evaluation of the toxicity of 2 surfactants (alkyl lauryl sulfonate (LAS), lauryl dimethil (hidroxyetil) ammonium chloride) and 4 trademarks of detergent (Ariel, Foca, Roma and Salvo) was carried out in microalgae (Pseudokirchneriella subcapitata and Monoraphidium sp.), macrophytes (Lemna gibba and Egeria densa), cladocerans (Daphnia magna, D. exilis, Moina macrocopa and Simocephalus mixtus), ostracods (Cypris sp.) and fish (Danio rerio). Toxicity bioassays were made with duration of 48 hours for tests with cladocerans and ostracods, and 96 hours for tests with microalgae, macrophytes and fish. The organisms were exposed to five concentrations of each of the detergents in triplicate (0.1, 1.0, 10, 50 and 100 and/or 100, 200, 400, 800 and 1000 mg L^{-1}) plus one control, without toxic. Each test at least three times repeated. With the data obtained lethal concentration 50 (LC_{50}) was determined by probit method (Probit-EPA, version 1.5). and a comparison between the LC_{50} and confidence intervals was performed to evaluate the statistical significance of the differences between the different treatments with detergents. In toxicity tests were obtained significant differences in the response of species to detergents, P. subcapitata, Monoraphidium sp, D. exilis and Cypris sp. were the most sensitive species and Lemna gibba and Egeria densa macrophytes were less sensitive to these products. The toxicity of detergents containing enzymes was superior compared with detergents not have enzymes in its formulation. Due to the wastewater treatment is limited and often sewage with high concentrations of detergents are discharged directly to aquatic systems, it is important to know the potentially adverse effects of these compounds to propose appropriate measures to reduce the risk which involves their presence in aquatic environments.

MP263 Screening ecological impacts of environmental surface waters using cell-based metabolomics

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Anthropogenic chemicals are routinely detected in aquatic ecosystems downstream from wastewater treatment plants (WWTPs), industrial and agricultural operations, and numerous other sources. Various studies have shown that exposure to such complex chemical mixtures can produce adverse health effects in resident organisms. Metabolomics has the ability to identify sensitive biological impacts produced by such exposures and provide information related to the primary mode of action. To increase throughput, a cell-based metabolomics approach has been developed to identify biochemical indicators of chemical exposures for rapidly assessing environmental impacts to support regulators. In this study, we applied cell-based metabolomics to detect biological impacts of environmental surface waters collected in the St. Louis Bay area, one of 43 Great Lakes areas of concern (AOCs) designated by the U.S. Environmental Protection Agency and Environment Canada. The goal of this study was to evaluate the utility of cell-based metabolomics for rapid and low cost monitoring of biological activity of surface waters receiving treated effluent from a wastewater treatment plant (WWTP) at this AOC. To do so, we first developed a procedure for exposing zebrafish liver (ZFL) cells to media prepared with surface water samples. By analyzing the metabolite profiles of ZFL cells using nuclear magnetic resonance (NMR) spectroscopy and GC/MS, we characterized cellular responses of ZFL cells exposed to the surface waters and assessed the biological impacts of WWTP effluents at this site.

MP264 Metabolic Profiles of Intersex Largemouth Bass: Toward Development of a Non-lethal Biomarker

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Intersex fish have been increasingly reported in rivers and impoundments across the US and around the world. Factors associated with development of testicular oocytes in phenotypic males are most commonly linked to endocrine disruption via exposure to environmental estrogens. Reduced sperm fertility and motility have been reported in intersex fish so the potential exists for population level effects, yet very little is known about alterations of physiological pathways in intersex fish. Current methods to positively identify intersex fish are lethal, requiring sacrifice of large numbers of wild fish; however, recent advances in technology allow for the simultaneous examination of thousands of endogenous metabolites in body tissues, including some that can be collected non-lethally (e.g. blood, mucus). Identification of unique metabolic profiles in intersex fish may aid in identification of key cellular pathways and lead to development of biomarkers in intersex fish. We investigated metabolic profiles of 39 phenotypic male largemouth bass with testicular oocyte counts ranging from 0 – 164 (per longitudinal histological section) from an impoundment in the Piedmont region of Georgia. The relative abundance of several metabolites from the liver, gonad, mucus, and blood plasma was significantly ($p< 0.05$) correlated with the number of oocytes detected in testicular tissues, lending insight to the mechanisms of intersex induction in these fish, and showing promise for the development of a non-lethal biomarker approach. Development of non-lethal biomarkers would greatly enhance our ability to sample larger numbers of wild fish and allow for repeated sampling of individuals, increasing our understanding of biochemical mechanisms, spatial and temporal trends, causative factors and adverse effects of the condition on an individual and population level.

MP265 Biomarkers of exposure to contaminants in three catfishes of Colombia

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Catfishes are one of the most abundant fish groups in Colombia accounting for 36% of them. However, 44% of the vulnerable and threatened fish species of the country belongs to the order Siluriformes. To evaluate biomarkers of exposure to contaminants, a total of 112 specimens of three catfish species were sampled in three different geographical areas of the country during the rainy and dry seasons of 2013. Fish species included capitán de la sabana (Eremophilus mutisii) from the Bogotá River, capaz (Pimelodus grosskopfii) from Huila and striped bagre (Pseudoplatystoma orinocoense) from the East Plains. Heparinized blood samples served to quantify lead and micronuclei assay whereas plasma was used to measure cholinesterase (AChE and BChE) activities. Liver was frozen in liquid nitrogen after necropsy and used to obtain S-9 fraction for glutathion-s-transferase (GST) and ethoxyresorufin- ortho-deethylation (EROD). Blood lead (interval in µg/dL) was found in 49% of the sampled fish: capitán (n=15), 3.7 – 7.8, 14 out of 15, capaz (n=25), 3.6 – 9.5, 7 out of 25; and striped bagre (n=9), 3.6 – 8.2, 4 out of 9. No fish had micronuclei above the baseline normal values reported. As for AChE and BChE, capitán displayed a significantly lower activity as compared to capaz and striped bagre (anova, $p < 0.05$). Hepatic GST (µmols/mg protein/min) of the three species had significantly different activities among each other: capaz (n=50), 1036 ± 163 [a]; striped bagre (n=30), 736 ± 138 [b]; and capitán (n=23), 422 ± 155 [c] (anova, $p < 0.05$). No significant changes were found between rainy and dry seasons. As for EROD (pmols resorufin/mg protein/min), fish sampled during the rainy season had almost 2-fold EROD as compared to those sampled during the dry season: capaz 2.5 ± 0.4 (rainy), 1.1 ± 0.3 (dry); capitán 0.8 ± 0.18 (rainy), 0.4 ± 0.07 (dry); and striped bagre 1.3 ± 0.3 (rainy), 0.8 ± 0.2 (dry). Along with the biomarkers, water analysis revealed that Total Petroleum Hydrocarbons (TPH) had 5 ppm diesel equivalents in the Bogotá River and 2-5 ppm in the East Plains rivers region, which suggests likely causes for high EROD. In previous studies, organophosphates insecticides were detected in the Bogotá River which could clarify the low AChE /BChE activity in the capitán. The areas studied in this investigation have impacts from coal mining, crude oil extraction and pesticides used which could explain some of the findings of the present work.

MP266 Biomarkers in Gambusia yucatana muscle for the evaluation of PAHs in "cenotes" (sinkholes) of Yucatan, Mexico

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In Yucatan's peninsula, groundwater is the main water supply for human, agricultural and industrial activities. Ground characteristics make recovery and treatment of wastewater very expensive, and it is returned to the aquifer without any treatment. This situation has contributed to an increase of pollutants reports in Yucatan's peninsula aquifer. Polyciclic Aromatic Hydrocarbons (PAHs) are legacy pollutants that are persistent and potentially carcinogenic. This study aimed to evaluate biomarkers in the muscle of mosquitofish (Gambusia yucatana) and its relationship with the presence of PAHs in water and sediment from seven "cenotes" (sinkholes). CYP1A expression, Glutathione-S-transferase (GST) Acetylcholinesterase (AChE), total glutathione (GSH) and lipid peroxides (LPO) were measured in muscle. PAHs in water and sediment were quantified by solid-phase extraction (SPE) and gas chromatography-mass spectrometry (GC-MS). Results indicated seasonal and spatial differences in PAHs concentrations and biomarkers response. Higher concentrations of total PAHs were found in sediments during the dry season and in water during the rainy season. During the dry season, AChE was significantly inhibited in all sampling sites respect to control. Total GSH was higher than control in most of the sampled sites during both seasons. LPO was significantly different to control in only two sites during the rainy season. GST was different to control in few sites, especially during the rainy season.CYP1A was over expressed in many of the sampling sites, especially during the dry season. There was not a clear relationship between the concentration of total PAHs in water and sediment and the response of biomarkers. This could be explained because of the presence of many other pollutants that may also be contributing to the response. This study was financed by grant UNAM-DGAPA-PAPIIT #IA202416 and by the Faculty of Chemistry PAIP program.

MP267 Biomarker responses and organochlorine pesticide accumulation in Müller's clawed frog (Xenopus muelleri) from the Phongolo floodplain, South Africa

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The Phongolo River floodplain hosts to the highest biodiversity of all of South Africa's floodplains and is situated in a malaria risk area where vector control is applied through indoor residual spraying (IRS). Persistent organic pollutants (POPs) like organochlorine pesticides (OCPs) are prone to accumulation in aquatic environments, where their stability increases. Aquatic organisms thus have a greater risk of continuous exposure. Amphibians are considered good indicators of ecosystem health. Xenopus muelleri is a fully aquatic amphibian species with highly permeable skin, making this species a perfect fit as indicator for OCPs exposure in the floodplain ecosystem. Biomarker responses are used as indicators of sub-lethal (biochemical) stress and manifest much earlier than conventionally used observable effects such as behavioural- or morphological changes. An array of biomarker response assays were analysed in liver and muscle samples of frogs collected from four surveys over two consecutive high – and low flow seasons. Chemical analysis for 22 different OCPs was carried out on the remaining whole frogs. Results indicated γ-hexachlorocyclohexane (γ-HCH – lindane) and dichlorodiphenyltrichloroethane (DDT – and its derivatives) as the main contributing pesticides. The maximum total OCP concentration in a frog measured at 21,399.0 ng/g lipid. Oxidative stress responses, involving various antioxidant enzyme activity and oxidative damage assays, were observed to have correlations with OCP accumulation along with similar tendencies in acetylcholinesterase (AChE) and Cytochrome p450 (CYP450) exposure biomarkers. Changes in the cellular energy allocation (CEA) of the frogs also corresponded to OCP bioaccumulation. A significant increase in OCP concentrations was measured from 2013 to 2014 regardless of river flow fluctuations. Corresponding responses in oxidative stress and CEA were observed. These results indicated that amphibians accumulated OCPs from the aquatic environment and that biomarker responses corresponded to the presence of these contaminants in their tissue. Further investigation into these response correlations could have a significant impact on the tissue threshold levels for OCPs used in regulatory guidelines.

MP268 Biomarker and Fluorescence Induction-Relaxation Responses of Cultured Corals and Coral Larvae to Bunker and Diesel Oil, Copper and Temperature Stress

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The impacts of stressors such as pollution and climate changes on coral reefs is a global concern, and sensitive and non-destructive methods are needed to assess health of coral reef systems and symbiotic zooxanthellae. This study compared indices of coral stress as measured biochemical methods to Fluorescence Induction and Relaxation (FIRe) measurements, a tool to assess photosystem responses to stressors in the symbiotic zooxanthellae. In Phase I of this study, aquarium cultured corals from three species Acropora divaricata, Montipora capricornis and Caulastrea furcata wereexposed to Cu, pyrene or elevated temperature (24-28C) for 72 hours. FIRe measurements were compared to measures of oxidative stress, lipid peroxidation and glutathione concentrations. In a later study, larval bundles of Montipora capitata during spawning events at a reference site (Hawaii Institute of Marine Biology) and a moderately polluted site (Pearl Harbor Hawaii). The larvae bundles were exposed to concentrations of bunker C oil, marine diesel fuel for 10 hours. Following FIRe measurements, the larvae were analyzed for lipid peroxidation (MDA), glutathione (GSH), glutathione s-transferase (GST), superoxide dismutase (SOD) and ethoxyresorufin o-deethylase (EROD). FIRe proved more sensitive to some stressors than biochemical markers; at high levels of stress results were similar for both techniques.

MP269 Recovery and remaining environmental effects in receiving waters of Swedish pulp and paper mills

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This study, reviews, field studies and research conducted in Sweden during the last 50 years to determine the environmental impact of the pulp and paper industry on aquatic ecosystems. A dozen mills with various process concepts and geographical locations were chosen for in-depth studies of effluent characteristics and the environmental response to changes in effluent load over time. The effluents contain compounds capable to induce fertilising effects, as well as toxic substances causing exposure and health effects in fish. Before actions against water pollution were introduced in the late 1960s large oxygen depleted zones in the receiving waters were common due to high content of organic matter in the effluents and to substantial fibre loss. Organic mercury compounds were widely used as pesticides in the mill operations. Dioxins unintentionally formed when elemental chlorine was used as the main bleaching agent. A number of mitigating actions, such as the shift from sulphite to kraft pulp production, improved technical standard within the mills including modern bleaching techniques, black liquor recovery and effluent treatment have been vital to the recovery process. A substantially decreased load of organic matter and nutrients have led to improved water quality in terms of higher oxygen concentrations, increased Secchi depth and reduced phytoplankton production followed by a slower, but yet significant re-establishment of macrophyte, zoo benthos, and fish communities. In addition, strong reduction of various toxic substances in the effluents has resulted in a good recovery of the fish health status in the receiving waters and in decreasing levels of such substances in sediments and non-migratory fish species. However, remaining, but weaker, disturbances of fish health, e.g., delayed sexual maturity and deviations in physiological biomarkers, are still observed in some areas. As the effects of this kind are observed also in areas where mill operation has closed, historical sediment contamination may be a probable contributing factor. Another factor of importance is the morphometrical characteristics of the receiving areas. Areas with fast water turnover and bottom dynamic conditions unfavourable for sediment deposition have generally recovered faster. To conclude, the study shows that the ecosystem resilience and capability to recover from pollution has been strong, although, in some cases, the mechanisms behind remaining ecotoxicological effects are not fully explained.

MP270 PFOS Impact on Zebrafish (Danio rerio) Cardiac Development Through Gene Expression and Morphometrics

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Perfluoroalkylated substances (PFAS) are fully fluorinated carbon chains of different lengths with a charged functional group attached, such as carboxylic acid or sulfonic acid. PFAS are frequently found in commercial and industrial products for the purpose of resisting stains, oils, soil, water, and fire-proofing. PFAS have been widely used, from industry to household products. In previous studies from our lab significant effects on pericardial sac size were observed at doses of perfluorooctane sulfonate (PFOS) ranging from 0.02- 2 µM. In the current study we sought to explore the morphometric effects at lower doses (0.0001 µM, 0.001 µM, 0.01 µM, 0.1 µM, 1 µM). The 0.0001 µM group for morphometrics showed statistically significant difference in pericardial sac size as well as total body length. The 0.001µM group showed statistically significant difference in total body length. Zebrafish were also exposed to 2 µM PFOS for 120 hpf and through a specific extraction protocol, the larval hearts were extracted and tested through gene expression. A significant increase in gene expression was observed in, homebox protein (nkx2.5) and ventricular myosin heavy chain (vmhc) following exposure.

MP271 Effects of pyrene, 2-methylnaphthalene, and phenanthrene of early life stages of three aquaculture fishes in Korea

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Marine oil spill accidents give a variety of adverse influences on aquaculture organisms for a long time. Polycyclic aromatic hydrocarbons (PAHs), one of the major crude oil constituents, have been reported to induce developmental inhibition, morphological deformity, histopathological effects and genetic damage on fishes. In this study, we evaluated the impact of the PAHs on early life stages of 3 aquaculture fishes (red sea-bream, Pagrus major, rock bream, Oplegnathus fasciatus, and olive flounder, Paralichthys olivaceus) using pyrene, 2-methylnaphthalene, and phenanthrene. Embryonic development and larval morphology were investigated for each species. The median effect concentration (EC50) was not estimated for pyrene, and phenanthrene for all test species. The EC50s for 2-methylnaphthalene of Pagrus major, Oplegnathus fasciatus, and Paralichthys olivaceus were 12.8, 10.9, and 18.0 µmol/L, respectively. The no observed effect concentrations (NOECs) for pyrene of Pagrus major, Oplegnathus fasciatus and Paralichthys olivaceus were 99, 99, 0.013 µmol/L, respectively. The NOECs for 2-methylnaphthalene of Pagrus major, Oplegnathus fasciatus and Paralichthys olivaceus were 5.5, 5.5, and 3.5 µmol/L, respectively. The NOECs for phenanthrene of Pagrus major, Oplegnathus fasciatus, and Paralichthys olivaceus were 7.0, 224, 0.96 µmol/L, respectively. There was no morphological deformity in the hatched larvae of Pagrus major exposed three PAHs. The spinal curvature and fin deformity were observed in Oplegnathus fasciatus at 11 µmol/L of 2-metylnaphthalene. Pericardial edema, ocular development inhibition, spinal curvature and fin deformity were observed in Paralichthys olivaceus exposed to 0.013 µmol/L of pyrene, 3.5 µmol/L of 2-metylnaphthalene, and 1.0 µmol/L of phenanthrene. It was considered that Paralichthys olivaceus to be more sensitive than Pagrus major and Oplegnathus fasciatus to PAHs. Some embryo of Paralichthys olivaceus successfully hatched in lower concentrations, while morphological deformity was observed in these concentrations. PAHs seem to have large effects on the early life stages of aquaculture fishes.

MP272 Eco-Pharmaco-Stewardship: An Update

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The pharmaceutical industry recognizes and understands concerns raised by stakeholders regarding the presence of pharmaceuticals in the environment (PIE). The major source of pharmaceuticals entering into the environment is via patient excretion following use of a medicine that is taken to prevent, cure or alleviate a medical condition. A comparatively smaller contribution to PIE stems from emissions from industry during the manufacture of pharmaceuticals and from the incorrect disposal of unused or expired medicines. Industry is committed to playing a role in addressing concerns about PIE and is actively engaged in minimizing the impact of its activities on the environment. Founded on the principles of product stewardship, the Eco-Pharmaco-Stewardship (EPS) initiative has been developed. It considers the entire life-cycle of the medicine and addresses the roles and responsibilities of all parties involved, including public services, the pharmaceuticals industry, environmental experts, doctors, pharmacists, and patients. The EPS initiative is supported by three 'pillars', which have been identified as the initial key areas of focus for the pharmaceutical industry: Pillar 1 – IMI iPIE project: the identification of the potential environmental risks of existing and new active pharmaceutical ingredients (API) through intelligent and targeted assessment strategies. Pillar 2 – Manufacturing effluents management: the compilation of best industry practices, enabling manufacturers to minimize risks to the environment. Pillar 3 – extended ERA: the refinement of the existing environmental risk assessment (ERA) process for medicinal products to ensure that they remain up-to-date and relevant. The pharmaceutical industry stands ready and willing to work with other stakeholders in evaluating and working on all aspects of the medicines lifecycle that may have a harmful impact on the environment. In all of these endeavors, the key priority remains to ensure patients' access to medicines in cooperation with all stakeholders. The industry believes that the EPS approach can serve to address concerns and inform adequately the existing regulatory paradigm. This poster presents more details on EPS and progress made on these endeavors.

MP273 Trace Element Bioaccumulation in Multiple Snapper Species from the West Florida Shelf

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The Lutjanidae (snappers) are one of the most important recreational and commercial fisheries complexes in the Gulf of Mexico and play key roles in marine ecosystem function within tropical and subtropical waters worldwide. We analyzed the muscle tissue of 5 diverse snapper species (red snapper, Lutjanus campechanus; gray snapper, L. griseus; lane snapper, L. synagris; yellowtail snapper, Ocyurus chrysurus; vermilion snapper, Rhomboplites aurorubens) for two essential metals (Cu and Zn), three non-essential metals (Hg, Cd and Pb), one essential non-metal (Se) and one non-essential metalloid (As). Snappers (n = 601) were collected from West Florida Shelf offshore waters during the Florida Fish and Wildlife Research Institute's fisheries-independent research cruises and muscle tissue was analyzed for trace elements using microwave digestion and Inductively Coupled Plasma Mass Spectrometry (ICP-MS). All trace elements were detected in all snapper species examined. For the essential elements, Zn was found in highest concentration overall, followed by Se and Cu. For the non-essential elements, As was found in highest concentration, followed by Hg, Pb and Cd. Lane snapper contained the highest concentration of Hg (0.81 µg/g dry wt), with vermilion snapper containing the lowest (0.23 µg/g dry wt), reflecting differences in trophic position. All five snapper species had a Hg concentration below the 1 ppm FDA guideline for human consumption. All species contained relatively low concentrations of Pb, ranging from 0.008 µg/g dry wt in vermillion snapper to 0.078 µg/g dry wt in red snapper. Arsenic was highest in gray snapper (21.8 µg/g dry wt) potentially reflecting the estuarine linkage of this species during early life history stages. This is the first in-depth study to investigate the concentration of multiple trace elements in ecologically diverse snapper species. These results have implications for trace element exposure, trophic pathways, and inshore-offshore habitat linkages that are important for effective management of reef fish populations.

Environmental or Analytical Chemistry – Poster Only – Part 1

MP274 A Pilot Study on the Detection of Select Pharmaceuticals in Hospital Wastewater and Wastewater Treatment Plants (WWTPs) in Doha, Qatar

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Pharmaceuticals and personal care products (PPCPs) are considered as chemicals of emerging environmental concern in the aquatic environment worldwide. This study was carried out to optimize an analytical method to investigate and quantify the occurrence of eight selected antibiotics: Penicillin, Amoxicillin, Gentamicin, Ciprofloxacin, Tetracycline, Erythromycin, Metronidazole, Clavulanic acid and Caffeine in Hospital Wastewater (HWW), influents and effluents from two wastewater treatment plants (WWTPs) in Doha. The extraction method was based on the automated solid phase extraction (SPE). The analysis of antibiotics in wastewater samples was performed using liquid chromatography – mass spectrometry (LC-MS) with positive ion electrospray ESI (+). The results indicated that antibiotics and Caffeine were widespread pollutants in wastewater. Caffeine exhibited high prevalence (> 100 ng/mL) in HWW and influent samples collected from both Old (OWWTP) and New (NWWTP) wastewater treatment plants. The highest concentration of Metronidazole and Ciprofloxacin were detected in the HWW at levels of 5.46 ng/ml and 1.99 ng/ml, respectively. In effluent samples of NWWTP, Clavulanic acid was the most prominent compound at concentration as high as 84.74 ng/mL. The highest concentration of Erythromycin (7.20 ng/mL) was detected in the OWWTP influent sample. Tetracycline was spotted at levels of ~ 0.2 ng/ml in most of the samples collected during the study. The levels of Amoxicillin, Penicillin, and Erythromycin were not detected in most of the samples collected from all collection points. There were a significant interaction between most of the studied compounds. The statistical analysis showed that there is no significant difference ($P \geq 0.05$) between OWWTP and NWWTP and the methods used in both WWTPs are effective in reducing/removing Caffeine, Penicillin and Metronidazole. The removal efficiency for Caffeine and Penicillin at the NWWTP was 99.44% on average, while OWWTP was effective to remove Caffeine and Metronidazole with removal efficiencies of 95.21% and 90.22%, respectively. These findings demonstrated that the LC-MS method optimized in this study is sensitive and selective to detect such low concentrations of PPCPs and the instrument is a robust and reliable tool that can be easily used in the routine analysis of compounds, like antibiotics, in complex matrices (wastewater).

MP275 A simultaneous detection of disperse dyes in wastewater textiles industry

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The massive use of dyes in the textile industry has raised concern with their wastewater. Untreated effluent can conveniently reach springs and other surface waters causing great damage to aquatic biota and uptake of water treatment plants because of their toxicity and mutagenicity. Their occurrence in these matrices is demanded but is complex due to the high dilution. Among them, the disperse dyes, strongly used in the dyeing of synthetic fibers have demanded attention due to high hydrophobicity, and difficult to be monitored by conventional analytical methods. Although their detection is of great importance in aquatic environments have high retention on reverse phase columns which make their separation exceedingly complex. The aim of this work present an analytical methodology for the detection and simultaneous quantification of disperse dyes widely used in the textile industry, such as: disperse orange 25 (DO25), disperse yellow 3 (DY3), disperse orange 1 (DO1), disperse orange 37 (DO37), disperse yellow 9 (DY9), disperse yellow 7 (DY7), disperse orange 3 (DO3), disperse red 1 (DR1), disperse red 13 (DR13), disperse red 60 (DR60), disperse orange 30 (DO30), disperse red 82 (DR82) and disperse blue 291 (DB291). The industry effluent samples were extracted and analyzed by LC-MS/MS using reversed phase PFP column and acetonitrile:water both containing 1% formic acid in gradient program. The simultaneous analysis of 13 disperse dyes has been validated in accordance with the rules and presented recovery of 70-99%, LD (0.05 – 1 ngL^{-1}), LQ (0.25 – 5 ngL^{-1}), linearity (0.999) and intra- and inter-days precision below 20%. The analysis of real samples confirmed by validation methods indicates the occurrence of DR60, DY3, DO1, DO37, DO30 in the matrices. Other dyes were detected, but not quantified, except for DO3, DY7 and DR13. Disperse dyes can be determined at low concentration by LC-MS/MS in industrial effluents with high precision and accuracy. Our findings show that dyes are present in efluents from textile industry and untreated river water which has been subjected to dyeing-textile discharges.

MP276 An Examination of Soil Microbial Biomass and the Impact of Seasonal Variation

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Soil microbial biomass is an important measure of the health and viability of a soil. It is also used to assess whether a soil is appropriate for testing under such guidelines like the OECD 307 Guideline 'Aerobic and Anaerobic Transformation in Soil' and the EPA Guideline OCSPP 835.4100 'Aerobic Soil Metabolism'. It is used for the same purpose for soil toxicity testing in the OECD 216/217 Soil Microorganisms: Nitrogen and Carbon Transformation Test Guidelines. These guidelines state that a well-established microbial population in soil and one that is appropriate for testing equals 1% of the soil's organic carbon content. This value is important for testing to ensure the soil is representative of soils in nature and can provide accurate degradation rates in soil metabolism studies and meaningful toxicity values in the nitrogen and carbon transformation (a.k.a., soil microflora) testing. A collection of initial soil microbial biomass values has been summarized and presented based on their time of collection during the year. Conclusions are extrapolated from trends in the data concerning seasonality and environmental conditions.

MP277 Assessment of Steroid Hormone Binding Affinity to Titanium Dioxide Nanoparticles in Aquatic Biome and Other Matrices

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Titanium dioxide nanoparticles (nTiO2) are prevalent in many products manufactured today, and the extent of their presence in the aquatic environment is at least 11,000 tons anually. These nanoparticles have unique physiochemical properties, and have been shown to gather in the gonads of fish upon exposure and potentially influence endocrine disruption. As titanium dioxide nanoparticles are used extensively in ingested and topical applications, it is important to understand how these nanoparticles are interacting with other endocrine disrupting chemcials (EDCs), potentially concentrating them in the gonads and acting as an agonist that compounds hormonal imbalance. The effects of endocrine disruption can be severe, leading to a range of maladies from neurological disorders to cancer and reproductive failure. This research will provide a direct correlation between nanoparticle-EDC interaction and reproductive abnormality using biomarkers capable of detecting endocrine disruption in its early stages. Understanding the manner in which titanium dioxide nanoparticles interact with endocrine disruptors would be a milestone in developing better detoxification treatments. The purpose of the research described in this presentation is to assess the impact of titanium dioxide nanoparticles on the model organism danio rerio (zebrafish) by monitoring reproductive failure. The ultimate goal is the complete characterization of nanoparticle behavior in the environment in order to conduct validated flow-through dosing studies in accordance with OECD guidelines. The role of biologics and endocrine disrupting chemicals in the environment were evaluated and molecular binding affinity was quantified using an analytical method based on pH-mediated capillary electrophoresis. With this technique, the impact of titanium dioxide nanoparticle interactions with biomolecules in aquatic systems can be explored, starting from pristine water samples to model protein mixtures, and finally in naturally occurring organic matter.The broader impact of this research will be to provide a holistic view of TiO_2 nanoparticle interactions in the environment to understand the complex role of nanoparticles in aquatic toxicity.

MP278 Bisphenol A removal from water and wastewater using Activated Carbon-alginate and Bentonite-alginate beads

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Bisphenol A (BPA) is a monomer commonly used as a plasticizer on several industrial processes. This compound is later released into the environment by the effluents of wastewater treatment plants (WWTP). It is classified as an Endocrine Disruptor Compound (EDC), which are chemicals of emerging concern that can block or mimic the activity of natural hormones, interfering with the reproductive systems of wildlife and humans. Currently, BPA is not regulated by the federal government in the US and wastewater treatment plants are not designed nor required to remove it. As wastewater effluent for drinking water use becomes a reality in our society, it warrants a need to develop methods to effectively remove BPA from wastewater. The objective of this project is to develop a novel sorption application in which we will incorporate bentonite and activated carbon with the gelling properties of alginate to develop alginate gel-based (hydrogel) adsorbent beads to remove BPA and provide a low cost, non-toxic alternative to the existing removal procedures, in which the preparation won't require nor release any hazardous byproducts. The removal capacities for BPA by these materials will be compared; factors, such as pH and loading, will be investigated to optimize the removal of BPA in water; and the adjusted absorbing conditions will be tested in wastewater for the possibility of future application in wastewater treatment plants or on drinking water filters.

MP279 Caffeine in an Urbanized Estuary: Past and Present Influence of Wastewater Effluents in Boston Harbor, MA, USA

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Caffeine has been identified by previous research as a potential tracer of sanitary wastewater. To further assess the utility of caffeine as a tracer of wastewater sources, samples from 25 sites throughout Boston Harbor were collected and analyzed for caffeine by LC-MS/MS. Caffeine concentrations in Boston Harbor ranged from 15 ng/L in the outer harbor to a high of 185 ng/L in the inner harbor; mean concentrations and median concentrations were 51 ng/L were 33 ng/L respectively. These data were visualized by a simple inverse distance weighting model to improve the understanding of transport and fate dynamics of wastewater derived contaminants. Elevated concentrations of caffeine in the inner harbor during the sampling period were determined to be the result of a combined sewage overflow (CSO) event as well as illicit discharge of sanitary sewage into municipal storm drains. A comparison of contemporary results to data from 1998 to 1999 shows significant reductions in caffeine levels within the harbor. For instance, concentrations were reduced by a factor of approximately 20 at the site of the former wastewater effluent discharge outfall in Boston Harbor. Lower present-day concentrations throughout the harbor were attributed to the relocation of effluent discharge from within the harbor to Massachusetts Bay, and a reduction in the number and discharge volume of CSOs. Spatial distributions of caffeine identified CSOs as the major contemporary source of contaminants to the inner harbor. These results provide additional evidence for the use of caffeine as an effective tracer of sanitary wastewater sources in urban estuaries. Furthermore, these results demonstrate the efficacy of both treated and untreated wastewater effluent reduction strategies put in place to improve the condition of Boston Harbor.

MP280 Characterization of municipal wastewater treatment plant effluent during major process upgrades

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The Region of Waterloo has several major waste water treatment plants (WWTP) which are currently undergoing major upgrades to improve effluent quality. These upgrades include improving the aeration systems, UV disinfection and decommissioning of the biosolids storage lagoons. Kitchener was a non-nitrifying carbonaceous activated sludge plant but after significant upgrades is now is fully nitrifying. Waterloo was a partially nitrifying conventional activated sludge plant prior to upgrades and is still partially nitrifying. Both treatment plants still have a number of major upgrades scheduled to come online in the next few years. Regular sampling of the effluent for a variety of emerging contaminants has occurred since 2010 and continued monthly from 2013-2015 during the implementation of major process changes. There has been a major decrease in total ammonia and increase in nitrate from the Kitchener WWTP which correspond to the treatment system upgrades (i.e. nitrification). In the Waterloo WWTP, the pattern of total ammonia and nitrate is much different, reflecting the recycling in the centrate from the biosolids back into the secondary train. The contaminant profiles, including selected pharmaceuticals, personal care products and estrogens, are contrasted over time. Concentrations of selected pharmaceuticals, such as ibuprofen and naproxen, are greatly reduced with increased treatment while other contaminants such as carbamazepine and venlafaxine remain recalcitrant. These improvements have potential significance for the environment downstream of the WWTPs where significant endocrine disruption has been documented, including high expression of intersex in fish.

MP281 Chemical Preservation of Semi-volatile PAH Compounds at Ambient Temperature: A Sediment Sample Holding Time Study

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Site investigations require the collection and analysis of representative environmental samples to delineate impacts, risk, and remediation options. When samples are removed from the environment, concentrations of semi-volatile polycyclic aromatic hydrocarbons (PAHs) begin to change due to various processes such as evaporation, adsorption, photo and microbial degradation. Preservation techniques are used to minimize these changes between collection and analysis. The most common techniques are refrigeration, freezing, and acidification. Regulatory agencies have developed holding time requirements of 14 days for PAH in soil/sediment samples stored at < 6°C. The technical basis for these requirements is not well defined yet failing to meet these criteria may deem the analytical results not valid. This study examined the effectiveness of using chemical preservatives for sediment PAH samples to defensibly extend the prescribed holding time requirements in the absence of refrigeration. Sediment samples collected at three (3) separate sites impacted with petrogenic and pyrogenic PAHs were analyzed as preserved (sodium azide) and unpreserved samples at defined time intervals up to 60 days. Statistical analysis of the data demonstrated no degradation of PAHs in the sediment samples preserved with sodium azide for up to 60 days at ambient or 4°C. Sodium azide preservation provides a useful technique to ensure sediment sample integrity in situations where maintaining holding temperature below 6°C during transport to the laboratory may be uncertain, e.g., during sample collection in remote locations.

MP282 Comparison of various digestion and extraction methods for the determination of heavy metals from soils polluted by electronic waste

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It is very essential to assess various digestion methods to accurately determine elemental concentrations in soils. In this present study, various digestion and extraction procedures were evaluated in order to assess the degree of contamination with heavy metals in soils polluted by electronic waste from Alaba International market, Lagos State, Nigeria.Total acid dissolution, aqua regia digestion, DTPA dissolution and 2M HNO3 extraction procedures were applied to samples.Ba, Cd, Cr, Cu, Ni, Pb, Sn, Zn and V were determined by inductive coupled plasma optical emission spectrometry (ICP-OES).A comparison of total acid dissolution including hydrofluoric acid (HF) treatment and aqua regia soluble fraction was done to estimate the applicability of aqua regia digestion in analysis of total metal concentrations in soils. The amount of Zn, Cu, Pb, Ni, Cd, Cr, V, Ba and Sn extracted by the four extraction methods showed a linear positive correlations which are statistically significant. Correlation coefficients between total metal concentrations and aqua regia contents revealed very strong positive correlations which are statistically significant (r values for Zn, Pb, Cd, Cr, Ni,V, Sn, Ba and Cu are 0.946, 0.973, 0.938, 0.901, 0.997, 0.956, 0.843, 0.982 and 0.930, respectively. These results showed that total metal contents, 2M HNO3 and DTPA can be useful preliminary indicator of areas where the risks of metal deficiency are high.

MP283 Concentration of Polycyclic Aromatic Hydrocarbons (PAHs) and Polychlorinated Biphenyls (PCBs) in urban and semi-urban soils in Havana

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Havana is the capital of the Republic of Cuba, with a population of more than 2 million citizens. In the last years more than one million tourists visited the city per year and with that also the traffic increased. Moreover, some industry is situated in the capital. Alongside with high human activities contaminants are emitted such as polycyclic aromatic hydrocarbons (PAHs) formed by incomplete combustion and polychlorinated biphenyls (PCBs). The main objectives of this study was to evaluate the level of these contaminants in the Havana province. Therefore, a soil monitoring was performed by the national center of animal and plant health (CENSA) in Cuba in collaboration with Agroscope ISS, Switzerland. The project was funded by the Swiss National Science Foundation and should also strengthen the analytical capacity of the CENSA laboratory. This will, in combination with the creation of a soil archive, increase the information flow between science represented by CENSA and the government represented by the ministry of science, technology and environment (CITMA) from Cuba. Soils were sampled at 12 agricultural sites, 1 industrial spot and at 7 parks in the Havana province. Concentrations of the sum of the 16 USEPA PAHs ranged from 0.04 to 71 mg Kg-1(dry weight, dw). The median of the agricultural soils was 0.1 mg Kg-1dw and the one of the 7 parks 1.9 mg Kg-1dw. The main source of pollution is pyrogenic with a predominance of diesel combustion produced by cars as an analysis of molecular PAH markers revealed. The sum of seven PCB congeners showed concentrations between 3 and 109 ug Kg-1dw. Different soil uses such as industrial, agricultural, or recreation had no significant influence on the PCB concentrations (p-value = 0.08). Some sites exceeded the trigger value of Switzerland for PAHs of 20 mg Kg-1dw according to that an eventual risk for human, animal and plant health needs to be assessed. As Cuba, up to now lacks environmental contaminant regulations this study serves as a basis to establish regulatory concentrations for organic contaminants. A long-term prospective is to extend the monitoring to other provinces and to the whole island to create a soil monitoring network. In parallel, the analytical laboratory capacity of CENSA was strengthened. Different extraction methods and chromatographic conditions were tested and the laboratory participated in a round robin to check the precision, accuracy and reliability of the results.

MP284 Determination of heavy metals in soils by microwave-assisted digestion and inductive coupled plasma optical emission spectrometry

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The analysis of the total concentration and the leachable fraction by aqua regia digestion of six heavy metals (Cd, Cr, Cu, Ni, Pb and Zn) in soils was determined by microwave digestion technique combined with the flame atomic absorption and inductive coupled plasma optical emission spectrometry. The acid mixture of HF-HCL-HNO_3 (1:3:1) was utilized to achieve total digestion of soils; the tests carried out provide evidence that the extraction of heavy metals from soils by the microwave-irradiated closed vessel system is a viable alternative to the traditional heating systems. The experimental study was conducted using two BCR standard reference materials BCR 141R and BCR 701 standard reference material. Maximum recovery, precision and accuracy established the excellent performance of the methods used.

MP285 Development of a simultaneous analytical method for agricultural chemicals in tap water using LC/MS/MS

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In order to ensure the safety of drinking water, many agricultural chemicals are monitored by many of water suppliers in Japan. However, analytical methods for agricultural chemicals in tap water are complicated. Therefore, much labor and cost is required to apply these methods. We have developed a simultaneous analysis method of 140 agricultural chemicals which are the "Complimentary Items" in tap water in the Japanese Waterworks Act, by liquid chromatography – tandem mass spectrometry (LC/MS/MS). Further, we evaluated the validity of the analytical method based on the results of the recovery tests of these agricultural chemicals added in tap-water at two concentrations (i.e., between 1/100 and 1/10 of the "desired value" and lower than 1/100 of the "desired values"). Limit of quantitation, repeatability precision, accuracy (recovery), and reproducibility precision (precision between laboratories) of each agricultural chemical were assessed based on the criteria in the guideline for validation of testing method in drinking water, which has been released by the Ministry of Health, Labour and Welfare. Regarding the 105 agricultural chemicals, the quantification limits were lower than 1/100 of the desired values of each agricultural chemical, and the accuracy (recovery) and repeatability satisfied the criteria of the above guideline. Regarding other 12 agricultural chemicals, the quantification limits were lower than 1/10 of the desired values of each agricultural chemical, and the accuracy (recovery) and repeatability also satisfied the criteria. Therefore, we judged that the simultaneous analytical method we have developed in the present study was applicable to the analysis of 117 agricultural chemicals in drinking water. In order to expand this LC/MS/MS simultaneous analysis method as a standard analytical method of drinking water, we will conduct the validity test of the analytical method among other examination organizations.

MP286 Development of Analytical Methodology UAE-HS-SPME for Organochlorine Pesticides Analysis in Water using Eva Passive Sampler's by GC-MS/MS

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Passive sampling is a method used for the monitoring of a wide range of xenobiotic compounds in environmental, such as pesticides. Organochlorine pesticides (OCPs) are highly toxic compounds and they are found in very low concentrations in the environment. Passive sampling emerges as a tool able to detect analytes in environmental samples. In order to improve the sensitivity and selectivity, we show an extraction method for passive sampling using a coupled analytical methodology between ultrasound assisted extractions (UAE)-headspace solid phase microextraction (HS-SPME). Analytical methodology UAE and HS-SPME were optimized by univariate analysis and central composite design, respectively. Analyses were performed using gas chromatography–mass spectrometric (GC-MS/MS) in the Multiple Reaction Monitoring (MRM) mode. The optimized methodology was able to obtain detections limits 0.322 – 0.516 µg L^{-1}, acceptable repeatability with a relative standard deviation less than 20% (n= 4) and recovery percentage >60%. External standard calibration was employed for the quantification, where linear ranges (from 1.0 to 5.0 µg L^{-1}) with r^2 values between 0.9902 to 0.9961 were observed. Finally, coupling the UAE, HS-SPME and GC-MS/MS suggest a methodology for the analysis of twenty organochlorine pesticides detected by passive sampler devices and it applied for their calibration in situ with total analysis time four hours and detection limits less than 0.5 µg L^{-1}. Acknowledgements: CRHIAM/ Conicyt/Fondap N° 15130015; Fondecyt N° 1140466 project

MP287 Effect of acid pretreatment on anaerobic biodegradation of rice straw

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Our study focused on both treating immense organic wastes (i.e., agricultural waste, sludge from WWTP) and obtaining useful energy resources like methane gas. Vegetable biomass such as rice straw is consist of non-biodegradable lignin, merely biodegradable cellulose and hemicellulose. Physical (i.e., grinding, heating, boiling), chemical (i.e., acid, base), and biological (i.e., microbe, fungi) pretreatment can be used to enhance hydrolysis efficiency and to extract available/useful carbon sources. Hemicellulose are known be degraded by acid pretreatment which has a relatively short reaction time and high sugar production yield. This study analyzed biologically producible methane gas and verify the acid pretreatment efficiency on anaerobic biodegradation using biochemical methane potential (BMP) test. Acid pretreatment was conducted using 2% H_2SO_4 with 1:10 (solid:liquid) ratio and autoclaved (121?, 60 min) for accerelating hydrolysis process. Four types of substrates were used for the experiment, which are (1)untreated rice straw, and acid-pretreated rice straw in phase of (2)solid, (3)liquid, and (4)solid/liquid phase. The produced methane amount using liquid phase was expected to be significantly higher than other substrates since the carbon sources in liquid phase are mainly short sugar chains, while carbon sources in solid phase are mainly lignin, cellulose, hemicellulose and some sugars. However the methane produced amount was similar between liquid phase of acid-pretreated rice straw (434 mL CH_4/g TC) and untreated rice straw (solid phase, 430 mL CH_4/g TC) indicating that the methane production reaction in liquid phase was inhibited due to the residual salts. Acid pretreatment using strong acid might have significantly reduced bioavailable fraction of rice straw, therefore different conditions of acid pretreatment will be applied in order to verify the optimal condition of acid pretreatment in anaerobic biodegradation.

MP288 Enantiomerization of Metalaxyl in South Carolina Soils

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Many pesticides on the market today are chiral, which means that they contain two enantiomers. Enantiomers are molecules that are mirror images but are non-superimposable. These pesticides are usually sold as a racemic mixture, which contains equal amounts of both enantiomers; however, typically only one enantiomer is toxic to the target pest. In an effort to decrease the mass of pesticides in the environment, manufacturers could market only the most effective enantiomer of the pesticide. However, if the single enantiomer is unstable in the environment (undergoes enantiomerization), it defeats the purpose of producing a single enantiomer formula. Little research has been conducted to determine how pesticides undergo enantiomerization in the environment, especially in soil systems. Metalaxyl is a systemic fungicide used in various crops that is marketed as a single enantiomer, metalaxyl-M (97% R-(-)-metaxyl and 3% S-(+)-metaxyl). Soil was collected near Lake Hartwell in Anderson, SC, and was analyzed for its mineral composition, pH, and organic matter content. Racemic metalaxyl and metalaxyl-M were exposed to the soil to study the change in enantiomeric fraction. The change in enantiomeric fractions in the presence of the soil was determined via HPLC. Preliminary data for metalaxyl in contact with individual minerals (e.g., calcite) showed a slight, but statistically significant change, in the enantiomer fraction. Similar behavior is expected for metaxyl in contact with the soil. In conclusion, by examining the effects of different types of soils and conditions (e.g., pH) on the rate of enantiomerization, more sustainable pesticides can be developed and manufactured. It may also help determine enantioselective degradation and the degradation products of these compounds, which can also be beneficial to toxicology studies and risk assessment.

MP289 Environmental Fate Studies with 14C-POEA

M. Kurtzweil, M. Shepard, Monsanto Company

Tallow amine polyethoxylate, commonly referred to as POEA (polyoxyethylene alkyl amine), is a nonionic surfactant contained in many glyphosate-based formulations that improves uptake and efficacy of the active ingredient, thereby reducing the amount needed for effective weed control. Four regulatory-guideline environmental fate studies were initiated in 2012 to obtain data for potential use in ecological risk assessments for formulations containing POEA and include: hydrolysis, aerobic aquatic degradation, adsorption/desorption, and aerobic soil degradation. ^{14}C-Labeled POEA was synthesized for use in the studies to aid in analysis of the surfactant, to assess mineralization to CO_2, and to allow for mass balance determinations. POEA was found to be chemically stable under abiotic hydrolysis conditions. POEA dissipated rapidly from the water column of natural water-sediment systems under aerobic conditions through a combination of metabolism and adsorption to sediment, with a DT_{50} of 2-3 hours. The rate of dissipation of POEA from the water column is consistent with rates reported in other water-sediment studies. Results from the adsorption/desorption study showed that POEA was strongly sorbed to soils and that sorption primarily correlated with increasing organic carbon. Based on the calculated adsorption coefficients, POEA is considered hardly mobile in soil according to the FAO mobility classification. Dissipation in both soil and sediment involved a combination of metabolism and binding. The DT_{50}s for POEA in the aerobic soil study ranged from 20 to 166 days with rates slowing as the percent organic carbon of the soils increased. Dissipation of POEA in the aerobic aquatic sediments was relatively slow and approximately 17-18% of the applied dose was still extractable at the end of the 100-day study in both systems. Based on results of the studies, along with predicted environmental concentrations and available toxicity data, acute and chronic risk to organisms in the water column and in the sediment/soil are considered to be low due to rapid dissipation/degradation of POEA and limited bioavailability.

MP290 Environmental Screening of Water Samples Utilizing Ion Mobility Enable High Resolution Mass Spectrometry

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Companies and environmental regulatory authorities are under pressure to develop screening methods capable of detecting a broad spectrum of environmental contaminants in a single analytical run. Many are turning to High Resolution Mass Spectrometry (HRMS) as part of the solution for obvious reasons since modern non-targeted, data independent, analyses offer a high level of selectivity through the acquisition of full spectra with accurate mass and isotopic information for both precursor and product ions in a single run. Following standards analysis, local lake and tap water samples were collected and extracted for PFASs. Data was acquired using alternating high and low collision energy states across the full analytical mass range, such that product ions were also generated. Ion mobility separations were performed simultaneous allowing the data be aligned by retention time and drift time giving extra selectivity. Multiple chromatographic and ionization techniques were used on a single platform to cover a wide range of compound classes. With a focus on perfluorinated compounds in water samples, information rich datasets were collected using data independent acquisition of both high and low energy simultaneously with ion mobility separation. The same instrument was operated in this mode coupled with a variety of chromatographic techniques and ionizing methods (including ESI and API). By utilizing the different chromatographic and ionization techniques the range of compounds covered can be expanded. Using a fully integrated scientific information system, which performs data processing via Apex 3D peak picking and componentization, a target list of compounds was screened against. In order to identify target compounds against a library mass error, isotopic fidelity and fragment matching was used. A unique measurement that is made via the mobility separation collisional cross section (CCS) giving an extra point of confirmation for known compounds. The mobility separation also allowed the spectral clean up in data allowing identification to be performed with more confidence. The same data was then used to isolate non-targeted compounds by using an array of comparison and discovery tools without the need to reprocess. Identification of these significant compounds of interest is also addressed by discussing elucidating techniques, such as a novel batch elucidation tool, available within the integrated scientific information system.

MP291 Evaluating PCB Congener Water Sample Contamination from Sampling Equipment

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This study evaluates the potential for sampling equipment to cause contamination in water samples analyzed for low level polychlorinated biphenyls (PCB). Equipment blank samples collected for previous County studies suggest that autosampler equipment may be contributing select PCB congeners to water samples at concentrations high enough to affect total PCBs results. Two sampling methods were used to collect surface water samples from the Green River in King County, Washington to better understand specific PCB congener contamination from autosampler equipment and evaluate the potential bias to Green River surface water samples collected in previous King County studies. Concurrent samples, one with an autosampler and one using hand-composite grab method, were collected at two locations under both baseflow and storm conditions. In addition, the study evaluates which piece(s) of the sampling equipment may contribute PCBs to the equipment blank contamination. Equipment samples included laboratory water that had passed through one of three types of sample tubing (silicone, Teflon®, and sampling splitting tubing) as well as equipment blank laboratory source water to evaluate potential PCB contamination from these materials. All samples were analyzed for PCB congeners using Method 1668c. The results indicate a very high bias in total PCBs in Green River water samples collected by the autosampler from PCB congeners 47, 51 and 68; on average they contributed 90% of the total PCBs in the samples collected with autosampler compared to 4% in hand-composite grabs. These same three congeners were detected in relatively high concentrations in Versilic SPX-50 silicone tubing equipment samples but not in the platinum-cured silicone tubing equipment samples. The Teflon® and sample splitting tubing showed very low detections of these congeners. The data from this study will be used to better understand the magnitude of equipment contamination bias and possible data correction approaches to total PCB congener results collected with autosamplers from previous County Green River studies as well as what modifications to sampling methods should be made for future water sampling efforts for PCB congeners.

MP292 Identification of Metabolites in Soil and Water-Sediment Studies Conducted with 14C-POEA

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Tallow amine polyethoxylate, commonly referred to as POEA (polyoxyethylene alkyl amine), is a nonionic surfactant contained in many glyphosate-based formulations that improves uptake and efficacy of the active ingredient, thereby reducing the amount needed for effective weed control. In addition to assessment of the dissipation rates of ^{14}C-POEA in soil and water-sediment degradation studies initiated in 2012, metabolites were identified in soil as well as in water and sediment. Metabolism was extensive as evidenced by the formation of $^{14}CO_2$ in both studies; ranging from 12 to 47% in the aerobic soil study and 10 to 15% in the aerobic aquatic study. Metabolism in the aerobic soil study was complex where four main classes of metabolites were identified by LC-MS and confirmed using a derivatization approach. All of the metabolites result from oxidative processes. The classes of metabolites consisted of tertiary amines containing both polyoxyethylene groups on nitrogen and terminally carboxylated aliphatic chains, secondary bis-polyoxyethylene amines and their corresponding mono- and di-carboxylate analogs, primary mono-polyoxyethylene amines and their corresponding carboxylated analogs, and di-carboxylated versions of polyethylene glycol. Metabolism in the aerobic aquatic study was not as complex as in the aerobic soil study as only tertiary amine metabolites containing both polyoxyethylene groups on nitrogen and terminally carboxylated aliphatic chains were identified as well as secondary bis-polyoxyethylene amine metabolites. This presentation will focus on discussion of the techniques used to identify metabolites in the aerobic soil and aerobic aquatic studies as well as the proposed pathways for metabolism of POEA in the two studies.

MP293 Implications of chronic carbon black exposure on the development of type 2 diabetes mellitus

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Type 2 diabetes mellitus is one of the most frequently diagnosed diseases in the modern world. Often, high fat diets and sedentary lifestyles are considered the cause of type 2 diabetes; however, recent studies have associated chronic exposure to particulate matter less than or equal to 2.5 micrometers in diameter with the development of key aspects of metabolic syndromes that eventually develop into type 2 diabetes. While pm2.5 has been studied extensively, the individual components it can be composed of have not, one of which being carbon black. Carbon black has been associated with cardiovascular disease and has also been shown to have some of the same metabolic syndrome aspects as pm2.5 such as disruption of AMPK pathways, gluconeogenesis, and cardiovascular functionality. The purpose of this study is to assess the impact of chronic exposure to carbon black particulates on human health related to type 2 diabetes mellitus through analysis of biochemical endpoints such as interleukin 6, glucose sensitivity, and liver accumulation. Study portions include an epidemiological study analyzing the diagnosis rates of type 2 diabetes mellitus in specific geographical zones with high levels of carbon black, air particle studies from varying regions to analyze the particle type and structure, and a full body chronic inhalation mouse study utilizing the particle types identified during the air particle studies through assessment of key biological endpoints associated with metabolic syndrome and development of type 2 diabetes mellitus.

MP294 Importance of Data Quality Management for Risk Assessment

C. Julias, CDM Smith

Data quality management (DQM) is the most critical part of an investigation and should be applied to every stages of the investigation, especially a remedial investigation and feasibility study at a Superfund site. Starting from investigation planning, sample collection and analysis, to data reporting and evaluation, DQM should be rigorously, thoroughly, and diligently implemented in every steps to ensure the data used for evaluation, especially for risk assessment, are complete, accurate, and precise. The quality of environmental data is important in assessing risks to human health and the environment when exposed to contaminants and, consequently, making risk management decisions. Poor data quality provided to data users, especially risk assessors, will result in costly and unnecessary resampling, worst yet making wrong remedial actions. Environmental data management software, such as Scribe and Environmental Quality Information System (EQuIS™), are used to plan, track, and document sample collection and analysis. In addition, EQuIS™ also provides automation for electronic data deliverable checks and submissions. Subsequently, using these software will minimize errors and efforts and, thus, reduce cost. However, there are also some issues and challenges when using these software that users should be aware. Using these software coupling with effective DQM will ensure the quality of data collected and analyzed at a contaminated site, especially at a Superfund site. Thus, a technically sound and legally defensible risk management decision can be made.

MP295 Interaction of BDE-47 and its Metabolite 6-OH-BDE-47 with the Human ABC Efflux Transporters P-gp and BCRP: Implications for Human Exposure Assessment

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ATP binding cassette (ABC) transporters, including P-glycoprotein (P-gp; MDR1) and breast cancer resistance protein (BCRP) are membrane bound proteins that mediate the cellular efflux of xenobiotics as an important defense against chemical toxins. Exposure to various environmental chemicals including brominated flame retardants, such as polybrominated diphenyl ethers (PBDEs), can result in changes in transporter gene expression; however, little information exists on how PBDEs and their metabolites interact with ABC transporters. The purpose of this study was to assess the interaction of BDE-47 and its hydroxylated metabolite 6-OH-BDE-47 with P-gp and BCRP using MDR1- and BCRP-expressing membrane vesicles and stably transfected NIH-3T3-MDR1 and MDCK-BCRP cell lines. In P-gp membranes, the parent compound BDE-47 had no measurable effect on P-gp activity; however, 6-OH-BDE-47 was a potent P-gp inhibitor (IC_{50} = 11.7 µM). In BCRP membranes, BDE-47 inhibited BCRP activity; however, 6-OH-BDE-47 was a stronger inhibitor [IC_{50} = 45.9 µM (BDE-47) vs. IC_{50} = 9.4 µM (6-OH-BDE-47)]. ATP-dependent transport of BDE-47 and 6-OH-BDE-47 was not detected, suggesting that neither compounds are substrates of P-gp or BCRP. Intracellular concentrations of known P-gp and BCRP substrates [(^{3}H)-paclitaxel and (^{3}H)-prazosin, respectively] were significantly higher (indicating less efflux) in both NIH-3T3-MDR1 and MDCK-BCRP cells in the presence of 6-OH-BDE-47, but not BDE-47. Collectively, our results indicate that the BDE-47 metabolite 6-OH-BDE-47 is an inhibitor of both P-gp and BCRP efflux activity. These findings suggest that some of the effects previously attributed to BDE-47 in biological systems may, in fact, be due to 6-OH-BDE-47. Implications for xenobiotic efflux and considerations for human exposure and risk assessment are discussed.

MP296 Legacy and current use pesticides: environmental relevance to wildlife reservationssy

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Agricultural practices and wildlife reservations are sometime located very close and the impact of pesticide applied are of great concern to those ecosystems mainly on sensitive species. The southern area of the Atlantic Forest is located in Misiones province, in the northwestern of Argentina. Misiones has more than 50% of its surface covered by national and international reservations, such as the Iguazú Falls (7 Wonders of Nature) and Yabotí MAB-UNESCO Reserve. Current-use and banned pesticides were measured in streamwater, suspended particulate matter (SPM) and bottom sediments (BS) in Acaraguá and Ramón rivers. These rivers are mainly used for direct consumption and domestic use by population. Samples were collected from upper, middle and lower sectors in each river, which run along agricultural lands passing through natural reservations. Endosulfans (a-, b- and sulfate), chlorothalonil, chlorpyrifos and trifularin were determined by GC-ECD. Pesticides more frequently detected were endosulfan and chlorpyrifos in water, SPM and BS, with high levels in streamwater (10-1700 ng/L) and the low in BS (0.2-1.3 ng/g dry wt). Chlorothalonil and trifluralin were only found in streamwater of Acaraguá (average 0.1 and 0.3 ng/L) and Ramón (average 10.4 and 0.5 ng/L) rivers. The a-/b-endosulfan ratios ranged from 3 to 10, showing a fresh application despite it was banned in 2013. Levels of endosulfan and chlorpyrifos in water were higher than the national limits for freshwater biota protection ≤7 ng/L a- + b-endosulfan and ≤6 ng/L for chlorpyrifos. Moreover, the abundant precipitation in this area (>2000 mm/year) enhanced the availability of both pesticides, facilitated by its relative hydrophilicity. Therefore, endosulfan and chlorpyrifos occurrence deserve more attention, and monitoring programs are recommended in order to diminish their incorporation to aquatic biota and, consequently to human. This study reveals the impact of agricultural use on wildlife reservations, which would be vulnerable to runoff of current and legacy pesticides. Studies involving atmospheric transport of these contaminants are now developing in our laboratory in order to understand the pesticide dynamics in these environments.

MP298 Predictive Modelling of Trace Metals Concentrations

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The input of trace metals from oil sands mining operations has led to a growing concern about water quality in the Athabasca River watershed of Alberta, Canada. These metals are derived from a variety of sources including those associated with oil sands mining, e.g., airborne releases from combustion, the bitumen upgraders, and wind-blown dust. Ground water, surface water overflow, and bank erosion are additional sources of metals to rivers and lakes in the oil sands area. These metals may, on occasion, exceed CCME water quality guidelines for the protection of aquatic life. Detection of trends in metal concentrations (and causal factors) can be challenging due the complexity of rivers resulting in spatial and temporal variability in metal concentrations, and interactions between different tributary sources such as groundwater, overland flow, and precipitation. Using data collected by the Regional Aquatics Monitoring Program (RAMP) over 1999-2014, we develop a method to predict trace metal concentrations in water using proxies including DOC (dissolved organic carbon), alkalinity, and TSS (total suspended solids). The efficacy of this model is demonstrated for the Athabasca River, the Clearwater River, and the east and west bank tributaries. In doing so, we characterize their unique chemical features, improving our understanding of the variability of metal concentrations in the oil sands area as a whole. This allows for improved investigations of temporal and spatial variations in metal concentrations and other aspects of the water chemistry within the watershed including how they vary with proximity to oil sands developments. Moreover, these predictive models provide an alternative method (to simple considerations of concentration) for investigating trends in trace metal concentrations by the inclusion of easily measured influencing variables. Additionally, our methods could be generalized to other watersheds.

MP299 Preliminary results of organochlorine pesticides in the atmospheric aerosol around Mexico using passive samplers

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The transport, persistence, bioaccumulation and biomagnification of organochlorine pesticides (OP) have made them of special interest; they also exhibit carcinogenic, mutagenic and toxic properties. OP in remote areas can be collected by passive samplers. In our study, we employed polyurethane foam (PUF) disk as sorbent in exposition through 90 days on 2014 (n=8) and 2015 (n=20) in five sites around México Country. Sampling sites are part of the Network of Atmospheric Observatories (RUOA) from Universidad Nacional Autonoma de Mexico. OP were extracted from the sampling media using dichloromethane in a system that consists of a homemade glass cell carrying PUF sample, it is coupling to reflux and immersed in an ultrasonic bath with time, potency and temperature control. The extraction variables were evaluated previously using a factorial design (2^3) obtaining the best conditions (50 °C, 40 % and 40 min, twice). Alpha-endosulfan was found in all sites, except at the east of the country (Los Tuxtlas, protected natural area 530 m.a.s.l.). Other OP in minor concentration was 4,4'-DDE. Nowadays, we are analyzing 60 samples; we will show their seasonal and spatial trend of the found OP.

MP300 Quality assessment of selected borehole water samples around selected sewage sites and industrial effluent discharge points

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This study assessed the quality of selected borehole water samples located around sewage sites and industrial effluent discharge points. Water samples were collected from sewage sites located at Iwofe, Eleme, Choba, Rumuomasi, Trans-Amadi and Industrial Effluent discharge points at Gbaran, Forcados and Bonny in Rivers state Nigeria. Two locations adopted as Control were located at Eneka and Elelenwo all in the metropolis of Rivers state Nigeria. Results indicated that for borehole samples around sewage sites: Conductivity, Temperature, Total Dissolved Solids, Total Suspended Solids were significantly low ($p< 0.05$) and were within the permissible limits of WHO and NSDWQ at all locations except for Total Suspended Solids, Nitrates, Total Coliform, Dissolved Oxygen, Fecal Count at Trans-Amadi were significantly higher while colour at all locations were within the limits. The pH value was below that recommended by WHO and NSDWQ (6.5-8.5), thus indicating that the water from all the locations which is also a characteristic of the Niger Delta region, irrespective of distance between boreholes and septic tanks, was acidic. Copper, Zinc, Chromium and Nickel were significantly low and within the permissible limits of WHO and NSDWQ at all locations ($p>0.05$). Mean concentration of Iron was higher in Trans Amadi due possibly to corroded pipes. Microbiological parameters were significantly high indicating the presence of Fecal Count and Total coliforms at all locations except in Eneka and Elelenwo. Parameters investigated for water samples located around Industrial effluent discharge points were within WHO and NSDWQ permissible limits, higher Turbidity was observed in relation to Conductivity at Gbaran and Forcados due to the presence of dissolved gases in the groundwater. While Chemical Parameters: pH, Chloride and Total Hardness were within the WHO and NSDWQ limits at all the location except for Dissolved Oxygen, Biochemical Oxygen Demand and Nitrates that are significantly different ($p>0.05$) and higher at Bonny, Gbaran and Forcados. Heavy Metals such as Copper, Zinc, Chromium and Nickel were significantly low and within the permissible limits of WHO and NSDWQ at all locations. Iron was significantly high at Gbaran compared to Forcados. Total Coliforms and Fecal count were present in the water samples from Gbaran, Forcados and Bonny indicating the presence of disease causing microorganisms.TPH and PAH of the water samples were lower than the WHO/NSDWQ permissible limits in all the locations studied. Generally, some of the parameters studied fell within WHO and NSDWQ permissible limits while some parameters exceeded the recommended limits for drinking water in some locations. It is therefore important that conscious effort and measures be made to reduce the introduction of contaminants into the environment as these find their way into the ground water and eventually the food chain.

MP301 Safety evaluation and bioactivity of extracts of selected plant species used to treat sexually transmitted diseases in southern Africa

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Background: Sexually transmitted diseases (STD's) have a major impact on sexual and reproductive health worldwide. Each year, the World Health Organization (WHO) estimates 448 million new cases of curable STD's are diagnosed. As the emergence of drug resistance in STD's related micro-organisms and potential side effects demands the discovery of newer drugs, the exploration of newer anti-microbial substances from natural sources may serve as promising alternatives. Despite the implied potential and long history of use, the hazards associated with the consumption of plant extracts are rarely investigated. Some plant species are known to produce poisonous chemical that could impact negatively on the health of consumers. In this study, twelve medicinal plants used traditionally in the treatment of sexually transmitted diseases are investigated for bioactivity and safe use. Methods: Ethanol plant extracts and three isolated flavonoids were evaluated for their anti-microbial activities against selected pathogens associated with STDs (one fungi and three bacteria). To determined anti-inflammatory activities of the extracts and compounds, the inhibitory effect was measured on the pro-inflammatory enzyme, 15-lipoxygenase. The extracts and compounds were also investigated for their anti-HIV activity against recombinant HIV-1 enzyme using non-radioactive HIV-RT colorimetric assay, as well as cytotoxicity in vero monkey kidney cell lines in vitro. Results: Acacia karoo and Rhoicissus tridentata extracts indicated good antimicrobial activity with MIC values ranging between 0.4 and 3.1 mg/mL. Extracts of Jasminum fluminense, Solanum tomentosum and flavonoid 2 and 3 had good

anti-inflammatory activity with IC_{50} less than positive control quercetin (IC_{50} = 48.86 µg/mL). Acacia karoo and flavonoid 3 exhibited moderate HIV RT inhibition activity of 66.8 and 63.7% respectively. Rhoicissus tridentata and Terminalia sericea had the best RT inhibition activity (75.7 and 100%) compared to that of the positive control doxorubicin (96.5%) at 100 µg/mL. The extracts had IC_{50} values ranging between 70.83-133.3 µg/mL, compared to actinomycin D (IC_{50} = 0.009 µg/mL) positive control. Conclusion: The observed activities may lead to new multi-target drug against sexually transmitted diseases. The tested extracts had low cytotoxicity on vero kidney cell lines, indicative of their relative safety.

MP302 Seasonal and spatial dynamic of current-use pesticides (CUPs) in an Argentinian watershed

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The Argentinian Pampa region is the major agricultural zone, in which, the agricultural lands are strongly linked to surface waters. However, Argentina has not regulation for most of the current -used pesticides (CUPs) in surface water to protect the aquatic life. The objective of this work was to study the seasonal and spatial variations of CUPs in surface waters of “El Crespo” stream, and to determine the maximum levels reached to evaluate the possible impact on aquatic life. “El Crespo” stream is only influenced by farming activities, with intensive crop systems upstream (US) and extensive livestock production downstream (DS). It is an optimal site for pesticide monitoring studies since there are no urban or industrial inputs into the system. Water samples were collected monthly from October 2014 to October 2015 in the US and DS sites by triplicate using 1 L polypropylene bottles and stored at -20°C until analysis. The samples were analyzed using liquid chromatography coupled to a tandem mass spectrometer (UPLC-MS/MS). The most frequently detected residues (>40%) were glyphosate (GLY) and its metabolite amino methylphosphonic acid (AMPA), atrazine, acetochlor, metolachlor, 2,4-D, metsulfuron methyl, fluorocloridone, imidacloprid, tebuconazole and epoxiconazole, which are used in the crops cultivated in the area (i.e. soybean, potato, maize and wheat). Individual analysis showed that the herbicide GLY and its metabolite AMPA presented seasonal and spatial variations. The highest concentrations of GLY and AMPA were detected in US site during spring 2014 (2.09 ± 0.39 and 1.13 ± 0.56 µg/L, respectively) and in DS during summer 2015 (1.06 ± 1.02 and 0.20 ± 0.23µg/L). Comparing totalCUPs concentration between sites, a significant increase in UP site during spring 2014 (4.03 ± 0.43 µg/L) in relation to DS (1.54 ± 1.17 µg/L) was observed, may be due to pesticide applications during fallow and transport via surface runoff. Data generated in the present research could be used for evaluating the possible impact of pesticide mixtures on aquatic life and for regulation guidelines.

MP303 Seasonal and Spatial Variation of Persistent Organic Pollutants in Mexico City

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Persistent Organic Pollutants (POPs) are a group of halogenated synthetic compounds widely used in common applications as pesticides, dielectric fluids and flame retardants. A large number of compounds are prohibited or restricted by the Stockholm Convention due to their high toxicity, risk to human health and environmental pollution. A simple, fast and green methodology was developed to determine POPs in airborne particle matter using a micro scale cell and ultrasonic assisted extraction. POPs were obtained in an online extraction-filtration-evaporation system and analyzed by gas chromatography – mass spectrometry/negative chemical ionization. Application of this methodology allowed us to study temporal and spatial POPs trend in five urban sites in Metropolitan Area of Mexico City (MAMC) by collecting particles ≤ 2.5 µm ($PM_{2.5}$) in 2013 and PM_{10} durin 2014. Concentrations of POPs between 0.1 and 6.9 pg m^{-3} were founded in all sampling period. BDE-99 (pentabromodiphenyl ether), endosulfan and endosulfan isomers were found in all sites and seasons. Endrin aldehyde, dieldrin, DDE and other polybrominated diphenyl ethers (PBDEs) were also found but they were not uniformly distributed. Temporary homogeneous distribution of organochlorine pesticides sum was observed (Kruskal-Wallis, KW, p = 0.8). Polybrominated diphenyl ethers sum showed lower concentration in rainy season compared with warm and cold dry seasons (KW, p ≤ 0.02). Northeast and Southeast showed higher pesticides sum concentrations than Center, Northwest and Southwest of MAMC (KW, p ≤ 0.01). PBDEs sum was higher in Northeast respect to the rest of MAMC (KW, p ≤ 0.004). Spatial trends were explained by predominant wind trajectories while temporal variations were result of old emission and background concentrations. The presence of DDT, endrin, HCH and endosulfanan derivatives showed evidence of old emission or long range transport. Results showed evidence of POPs contamination in MAMC and for our knowledge this is the first time that POPs were determine in airborne particle matter in urban sites from Mexico.

MP304 Selenium Analysis in Surface Water using EPA Method SW7742

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This field study, conducted at the New River in eastern North Carolina, compared results from two different analytical methods for the measurement of selenium concentrations in brackish surface water. Environmental Protection Agency (EPA) methods SW6010 and 6020 are common analytical methods for selenium analysis. The use of these methods in brackish water has a higher detection limit, and can cause elevated results of selenium due to the high salt content of the brackish water. EPA method SW7742, with borohydride digestion of the sample followed by graphite furnace atomic absorption (GFAA), provides a detection limit of less than 1 microgram per liter (µg/L) and is more suited for brackish water. Surface water samples were collected at either low or high tide in 2012, 2013, and 2014 and analyzed using methods SW6010/6020. Concentrations ranged from 9 to 180 µg/L. Those results seemed questionable because there are no known site-related sources of selenium discharge to the New River in this vicinity. Therefore, additional surface water samples were collected at low and high tide in 2016 and analyzed using method SW7742. Concentrations were between 0.2 and 0.4 µg/L. Based on comparison of the results, Method SW7742 is the more appropriate method to use when conducting selenium analysis in brackish water.

MP305 Structural Elucidation and Environmental Risk Assessment of Candesartan Ozonation Products

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Candesartan belongs to the class of angiotensin II receptor antagonists which are used for the treatment of hypertension. During the last years, its release into wastewater due to human excretion has considerably increased caused by a steady growth of its prescription rate. Candesartan is only partly eliminated during conventional wastewater treatment und thus enters the receiving waters. It has been detected in surface water up to 1,100 ng/l. The use of advanced wastewater treatment technologies such as ozonation is currently tested to eliminate trace organic substances which are not sufficiently removed during biological wastewater treatment. During ozonation, trace organic compounds are usually not completely mineralized but transformed into oxidized products. The molecular structure of these transformation products is mostly unknown and it is unclear whether they induce an environmental risk. Here, we applied laboratory-scale ozone treatment to pure candesartan solutions in order to identify possible transformation products. LC-MS measurements including software-assisted peak detection were conducted after the ozonated samples had been enriched by means of solid phase extraction. LC-MS^n experiments were carried out to tentatively identify the transformation products' molecular structures. To assess their environmental behavior in silico calculations for logP and biodegradability were performed. Additionally, the acute luminescent bacteria test using Vibrio fischeri was used for toxicity testing of the samples before and after ozonation. A wide variety of transformation products could be detected. Most of them showed lower retention times compared to candesartan. Compounds with higher as well as with lower molecular weights than candesartan were detected. The poster presents a selection of transformation products for which a molecular structure could be proposed and the hereof calculated lopP and biodegradability values. The logP values and the lower retention times indicate an increased polarity compared to candesartan. Thus, the transformation products possess a lower bioaccumulation potential as well as a decreased tendency to adsorb onto sediments and particles when released into surface water. Biodegradability calculations result in a slight up to a distinct increase of degradability under aerobic conditions. First results of toxicity testing show no toxic effect for the initial solution as well as for the ozonated samples.

MP306 The Biogeochemistry and Long-Range Atmospheric Contamination of Antarctic Glacial Cryoconites

A. Mass, D. McKnight, Univ of Colorado at Boulder / Inst of Arctic and Alpine Research

The condensation of gases in cold environments and the semivolatile nature of many persistent organic pollutants (POPs) lead to a temperature-driven global distillation process in which atmospheric contaminants are deposited and accumulate in the cryosphere. This poleward transport leads to multiple environmental concerns including halocarbon-driven ozone degradation, contamination of glacial headwaters, and the biomagnification of lipophilic pesticides in Arctic food chains. Although long-term monitoring programs in the Arctic examine the transport and environmental fate of pollutants in the Northern hemisphere, there is a significant lack of comparable monitoring in Antarctica and the distribution of semivolatile contamination in the Southern hemisphere is far less understood. This study analyzed glacial cryoconite meltwater collected over two austral summer seasons (November- February) in the McMurdo Dry Valleys of Antarctica in order to characterize the seasonally variable biogeochemistry of glacial meltwater, identify long-range atmospheric contaminant deposition on Antarctic glacial surfaces, and examine the effect of cryoconite water chemistry on the fate of persistent organic pollutants. Preliminary identification of low-level organochlorine pesticides in cryoconite water may broaden the database of contaminant studies available to model semivolatile distillation in the Southern hemisphere.

MP307 Use of Radon to Determine Site-Specific Attenuation between Subslab Soil Gas and Indoor Air for a Vapor Intrusion Evaluation of VOCs

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Vapor partitioning or attenuation refers to the reduction in volatile chemical concentrations that occurs during vapor migration in the subsurface, coupled with the dilution that can occur when the vapors enter a building and mix with indoor air. The aggregate effect of these physical and chemical attenuation mechanisms can be quantified through the use of a vapor intrusion (VI) attenuation factor (AF), which is defined as the ratio of the indoor air concentration arising from vapor intrusion to the soil gas concentration at the source or a depth of interest in the vapor migration route. Because of the presence of occupied buildings directly above a groundwater plume contaminated with VOCs, a multiple line of evidence indoor air study was conducted in support of a Superfund site investigation. Paired subslab soil gas and indoor air samples were collected from an occupied residence and office building during three sampling events (summer, fall and winter). Samples were analyzed for radon and a targeted set of VOCs (cis-1,2-dichloroethene, tetrachloroethene, trichloroethene, and vinyl chloride). Site-specific AFs were developed for subslab soil gas to indoor air using the measured radon and VOC results from the paired samples. Radon was consistently detected in both subslab soil gas and indoor air compared to several of the targeted VOCs which were less frequently detected. As a result, radon provided a good surrogate for estimation of a site-specific attenuation factor for the VOCs (radon allowed for development of AFs for the two structures even when VOCs were not detected). Site specific AFs for the residence and office building using radon and the VOCs were compared to EPA default AFs. While the attenuation factor developed for the residence was very consistent with EPA's default value (and the attenuation factors reported in the USEPA national database), the attenuation factor developed for the office building was significantly lower. Building construction features as well as heating and ventilation systems likely account for the difference in the observed AFs. Recommendations for incorporating site-specific AFs in VI risk assessments are presented and discussed.

Integrated Environmental Assessment and Management – Poster Only

MP309 A Tier II Plant Exposure Estimation Tool

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The United States Environmental Protection Agency's (EPA) Office of Pesticide Programs Environmental Fate and Effects Division (EFED) is developing a replacement for the TerrPlant model, which is used to estimate pesticide exposures to plants inhabiting terrestrial and wetland habitats. Conceptually, this model considers pesticide transport via spray drift and runoff from treated areas into and onto adjacent non-target habitats. The new model, Audrey III, makes use of existing models currently employed for estimating exposure by EPA, including AgDRIFT, the Pesticide Root Zone Model (PRZM5), and the Variable Volume Water Model (VVWM). In Audrey III, the terrestrial exposure model is focused on a conceptual Terrestrial Plant Exposure Zone (T-PEZ), whose width is determined by the distance from the edge of field traveled by overland sheet flow and whose depth is determined by the plant root zone. Within the T-PEZ, exposure is estimated separately for loading of pesticide entrained in runoff and sorbed to eroded sediment, and pesticide deposited directly onto foliage by spray drift. For areas outside of the T-PEZ, exposure is estimated for pesticide transported via spray drift only. A separate wetland conceptual model in Audrey III is based on a conceptual Wetland Plant Exposure Zone (W-PEZ), and assumes the same surface area dimensions used for the "Standard Pond" in PRZM5 (10 ha field and 1 ha body of water) although different dimensions for depth are assumed. Similar to the T-PEZ, concentrations are estimated based on loadings from runoff, eroded sediment, and spray drift; however, the W-PEZ is modeled as two completely mixed compartments (variable volume water column and benthos) linked together via mass-transfer. The aquatic exposure model is also based on pesticide loading from runoff, erosion, and spray drift to a relevant aquatic plant habitat. Potential applications of Audrey III in risk assessment will be presented.

MP310 An all-in-one ecological risk assessment (ERA) tool: AIST-MeRAM

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Ecological risk assessment (ERA) of chemicals is a complicated procedure that requires enormous time and labor in collection and assessment of the hazard data and a high level of expertise in interpreting the risk. Furthermore, new assessment methodologies have been increasingly developed, making the shift from traditional impact assessment at the organism level to more ecologically relevant assessments such as "species sensitivity distribution (SSD)" or "population-level effect modeling". For these reasons, ERA of chemicals is considered "something difficult or impossible for those with little assessment experience and cumbersome or complicated for practitioners". AIST-MeRAM released on December

2014, is an all-in-one risk assessment English version tool that embedded with all the necessary data, the scheme of Chemical Substance Control Law (CSCL) of Japan, and the ERA methodologies from simple hazard quotients to more complicated modellings such as SSD and population-level assessment approach. Visit to webpage of http://en-meram.aist-riss.jp/ for the tool download and the detailed information.

MP312 Comprehensive Three Method Sampling Program for Refining Exposure Estimates and Pinpointing Sources of Lead in a Site along a Large Western US River

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Elevated lead concentrations have been identified in near-shore sediments and upland soils on a site situated on a large river in the western United States with a history of mining operations. Following a removal action near a former tailings impoundment and mill operation, a comprehensive sampling program was completed to gain a deeper understanding of the horizontal and vertical extent of lead concentrations in the area for use in evaluating risk to human health and ecological receptors associated with near-shore sediment and soil. The sampling was also designed to determine if lead concentrations could be attributed to local sources that have the potential to contribute to contamination on beaches along the river. This comprehensive program combined three different sampling methods used in conjunction with one another to better characterize lead concentrations: incremental composite sampling (ICS), portable X-ray fluorescence (XRF), and coring. Composite sampling following ICS methodology was used to obtain results representing the concentration of lead for each of the 16 decision units (DUs) in the study. We evaluated results from all DUs to understand the horizontal distribution of lead over the areas sampled. The portable XRF analyzer was used to obtain lead concentrations in sieved and dried sediment and soil samples collected from between four to thirteen discrete locations within each DU. XRF results were used to both evaluate the horizontal distribution of lead within DUs and to guide the field-based decision on where to conduct coring. Confirmatory analysis of a subset of XRF samples validated use of the XRF results. Core samples were collected from three different depths to understand the vertical distribution of lead at locations of interest within each DU. Our evaluation of results from the three types of samples collected in this study indicate that XRF was effective in identifying locations of interest (based on higher lead concentrations) for core sampling. In addition, we used site-specific data to adjust lead concentrations for bioavailability to be more representative of human health exposure. Taken together, these methods provide comprehensive lead exposure information and information about horizontal and vertical extent of lead.

MP313 Cooperative Approaches & Simplified Methodologies for Rapid Assessment: Natural Resource Damage Assessment; Human Health & Ecological Risk Assessment

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Environmental assessments of oil and hazardous materials often involve “Natural Resource Damages” and “Environmental Risk”, which often encompass complex data analysis, allocation, and decision making. As a result, trustees, potential response parties (PRPs), and other decision makers are faced with lingering schedules and elevated costs. In addition, these assessments are typically performed as distinct efforts and at different time frames throughout the project remediation, even though they generally employ the same data and similar analysis. Most importantly, the valuable natural resources that have been damaged and the associated environmental risks, are delayed for restoration and resolution, as they await allocation and settlement funds. As a result, the overall benefits to the resources and parties involved do not justify or outweigh the significant associated costs. However, cooperative approaches with simplified methodologies and rapid assessment tools have been developed to address complex environmental data issues, thus expediting a cooperative and simplified process. For example, natural resource damage assessment employed in the early stages of remedial design can be coordinated with risk assessment to synchronize the needs for data collection and monitoring. This avoids unnecessary resampling or duplicate data gathering, and fulfills both process requirements with a cooperative well-designed investigation and restoration design that achieves a faster resolution of the entire site’s liability. This presentation identifies and describes the available rapid assessment tools and cooperative approaches that are provided by NewFields to facilitate this expedited process. These tools allow clients and decision makers access to the most timely and valuable information to arrive at overall conclusions of natural resource damages and potential environmental risks at facilities. Thus, site management occurs faster and natural resources are restored sooner for ecological and human reuse, which is the critical common goal. Uncertainties are present within any simulation of the environment, however, cooperative simplified assessment approaches provide overwhelming schedule and budget reductions that achieve restoration sooner, thus trumping the greatest benefit for all entities.

MP314 Development of inter-species correlation estimation (ICE) based species sensitivity distribution (SSD) models for use in hazard assessment

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Web-ICE (Web-based Inter-species Correlation Estimation) is an internet-based modeling platform developed by USEPA to relate acute toxicity of a chemical for one species to that of another. ICE allows the user to input an acute toxicity value for a surrogate species and predict the effect value for many other species, thus potentially filling in data gaps for a variety of environmental assessment purposes. ICE has been used to explore relatively straightforward species to species relationships, generalized Species Sensitivity Distributions, and make predictions of safe concentrations for endangered or threatened species exposed to chemicals. With the recent publication of ICE models for algal species (Brill et al. 2016) a full array of potential models for algae, invertebrates and fish for aquatic hazard assessments are available. Development of SSDs is particularly interesting and herein we described an assessment of SSDs based on acute toxicity for a number of well-studied industrial chemicals which possess databases large enough to make comparisons of ICE model SSDs with true SSDs. We seeded ICE models with traditional algal, Daphnia, and fish toxicity data from industrial chemical databases to generate full SSDs and probe their utility, taxonomic drivers, and the potential influences of empirical test species choices on SSD outputs for use in hazard assessments. This poster presents newly developed ICE-based SSDs and will compare ICE predicted SSDs to empirical SSDs using multiple seeding options. The ability to use ICE as an extrapolation technique for risk assessment greatly enhances our overall dependence on animal tests as well as presents another mechanism to reduce uncertainty factors applied to minimal data sets.

MP315 Effluent Water Quality from Seven Wastewater Treatment Plants in Savannah, Georgia, USA

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The city of Savannah is located on the southeast corner of the State of Georgia, USA. Metropolitan Savannah and its suburbs all together have a population of over 450,000. There are seven wastewater treatment plants (Crossroads, Georgetown, President Street, Garden City, Tybee, Wilshire, and Richmond Hill) in Savannah which serve the daily domestic wastewater treatment needs of this population. Savannah’s storm water drainage systems are very well connected with the wastewater lines. All these plants have the capacity to accommodate additional storm run off during high rainfall events. Recently, there has been a push to recycle the treated sludge and treated water from these facilities for irrigation to lawns, gardens and/or golf courses. However, there have also been some serious concerns for reusing both treated sludge and treated water that may contain heavy loads of nutrients and other toxic metals. The main objective of this study was to evaluate the variations in the pH, salinity, dissolved oxygen, NO_3-N, and elemental levels of effluent water from seven wastewater treatment plants in Savannah, Georgia. Effluent waters were collected from seven wastewater treatment plants in Savannah, Georgia and analyzed for pH, salinity, dissolved oxygen, NO_3-N and various elements. The result of this study indicated that, all these treatment plants effluent water varied in pH, salinity, NO_3-N, and elemental composition. It is a matter of great concern that, despite stringent environmental regulations, there has been no improvement in wastewater quality for this time period. Therefore, careful consideration must be given in reusing the effluent waters for land application to avoid human exposure via plant uptake and subsequent consumption of agricultural products.

MP316 Environmental effects monitoring protocol development: three Brazilian river estuary case studies

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The Brazilian Program for Assessment of Water Quality (PNQA) has not yet established a standardized methodology for environmental effects monitoring, making it difficult to compare the results from different water bodies. Recent movements have shown the importance of integrating physical, chemical, and biological results for environmental health studies. Some countries like Canada, United States, Sweden, Finland, New Zealand and Chile have already adopted these parameters in environmental monitoring programs, even in some of their regulations. In Canada this type of monitoring was implemented in the mid-90s, as part of the federal program of Environmental Effects Monitoring (EEM). This EEM program is used as a scientific feedback tool to determine if the current regulations are sufficiently protective of local receiving environments. The present study aimed at validating and adapting the Canadian EEM program, in a pilot project in Brazilian estuarine ecosystems referred to as the Fish Guide Project. The EEM approach was used to assess the health of three estuaries of Espirito Santo State: the Benevente, Jucu and Santa Maria da Vitoria rivers. Three sampling points were selected in each river along a hypothesized contamination gradient. Fish and benthos studies were conducted during three sampling periods in 2014 and 2015 in different seasons (dry and wet). In parallel, water and sediment chemical parameters were analyzed. Three different fish species were chosen as bioindicators, Genidens genidens, Ophioscion punctatissius and Eleotris pisoni, with November representing their reproductive season. The fish index (GSI, HIS and K) and benthos community structure results showed that the greatest environmental effects were observed in sampling points located in the region next to the sewage discharge, reflecting the results of chemical analyses of water, sediment, and fish filets. The greatest environmental effects have been observed in the Jucu River and the best environmental conditions were found at the Benevente River. From this study it was possible to evaluate the health status of the 3 Brazilian estuaries and to publish the Brazilian environmental effects monitoring protocol in December 2015. Adopting this protocol is expected to maximize the effectiveness of environmental monitoring studies while reducing time and cost.

MP317 EPA's National Reassessment of Contaminants in Fish from US Rivers

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Multiple EPA offices collaborated to conduct a reassessment of fish contamination in U.S. rivers as part of the Agency's 2013-14 National Rivers and Streams Assessment (NRSA). This is the first national assessment of contamination in river fish that will generate probability-based data for analysis of trends in fish contaminant levels when compared to fillet concentration results from the 2008-09 NRSA. In 2013 and 2014, field teams collected one fish composite sample per site from a statistically representative subset of 361 river locations (5^{th} order or greater), 224 sites of which were assessed during the 2008-09 NRSA. The composite samples consisted of up to five similarly sized adult fish of the same species that are commonly consumed by humans. Aliquots of fillet tissue from the composite samples were analyzed for mercury (361 composites) and 13 perfluorinated compounds (PFCs), including perfluoroctanesulfonic acid (PFOS) (352 composites). Fillet samples from the 224 sites that corresponded to locations sampled during the 2008-09 NRSA were also analyzed for the full complement of 209 PCB congeners. All 361 of the fillet samples contained detectable levels of mercury, and all fillet samples from the 224 previously sampled sites that were analyzed for PCBs contained one or more congeners. All but one fillet sample from the 352 composites contained PFCs and 349 of those fillet samples contained PFOS. The concentration ranges measured for mercury, total PCBs, and PFOS are 8.6-1,070 ng/g, 0.06-4,700 ng/g, and 0.16-9.9 ng/g, respectively. The 2013-14 NRSA fillet results indicate that 26% of the sampled population of rivers had fish with mercury tissue concentrations that exceeded EPA's 300 ppb fish tissue-based water quality criterion for methylmercury, 49% of the sampled population exceeded EPA's 12 ppb cancer human health screening value (SV) for PCBs, and 10% of the sampled population exceeded the Minnesota Department of Health 40 ppb human health SV for PFOS.

MP318 extreme weather events in the Río de la Plata: Threats and risks in coastal cities

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The flooding of the Río de la Plata are generated by the phenomenon of sudestada. It occurs at any time of the year, 90% of sudestadas take place between April and December although stronger (with higher gusts to 30 knots and usually with rainfall throughout the region) occur from March to October. It is characterized by persistent, regular Southeastern strong winds, relatively low temperatures and generally with rainfall of varying intensity, although sometimes it can occur without precipitation. In the case of sudestada with rainfall, it is generated by the combined effect of two systems, one high pressure (located on the Atlantic Ocean, off the coast of central Patagonia) that transports cold air maritime origin to the east of the province of Buenos Aires, south end of Coast and South Eastern Republic of Uruguay, and low pressure system (located on the Central and southern Mesopotamia and the western region of Uruguay) that brings warm, moist air over the region. When depression deepens, Southeast wind circulation industry producing the sudestada intensifies. In this work records extreme events of floods in the coast of the Río de la Plata between 1900-2000 in Quilmes, Berazategui, Ensenada, Berisso and Magdalena, area with large percentage of territory in floodrisk . It is known that in recent years, the average sea level on the coast of Argentina and River Plate have increased considerably according our records. Among the causes of the increase are the increased rainfall and rised water flows contribution of Uruguay and Parana Rivers. The causes of extreme flood events in the selected coastal strip are: rainfall (57.1% of cases), rains with overflowing streams (28.6%), rain with wind (11.4%) and promotion webs (2.9%). In the event of floods between 1987 and 2007 on the coast of Río de la Plata there was a large amount of flooded areas with a total of 15,318 evacuees, at least a total of 16 dead, with flooding March 1988 more evacuations occurred (8500 people). This event features across the coastal strip as evacuees exceeded 55% of total evacuations in the period in Quilmes; for Berazategui, 90.1%; for the game of Ensenada, 32.8%; and Berisso, the proportion was much lower, reaching barely 3.2% of total cases. We conclude that in the Risk Management of extreme weather events, must be addressed actions to reduce vulnerability and exposure of populations and increase the resilience of cities to cope with extreme weather events.

MP319 Filing a Biotechnology Submission under Toxic Substances Control Act (TSCA) and Environmental Hazard Analysis

A. Muneer, USEPA

Have you created an intergeneric microorganism species and thought about using it for commercial or research purposes? Did you know that the US Environmental Protection Agency has an application process in which you can do that? Come learn what the TSCA Biotechnology Program is about, including Microbial Commercial Activity Notice (MCAN), TSCA Experimental Release Application (TERA), and Tier I and Tier II exemptions, and Biotechnology Test Market Exemption Applications (TMEAs) while learning about what environmental hazards intergeneric microorganisms pose.

MP320 Hazard assessment of the water-soluble fraction from a petroleum-based lubricating oil in aquatic organisms

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Oil and gas transport depends on vast pipeline networks and many compressor stations, where automated actuator valves pressurize and control the flow of the oil and gas. These valve actuators use hydraulic fluid as a lubricant, and leakage of the fluids has caused soil staining around the valves. Direct toxicity risks of this lubricating oil to aquatic organisms from groundwater infiltration as well as spring runoff are uncertain. Therefore, a hazard assessment of UNIVIS J13 lubricating oil was performed to evaluate the toxicity of the mixture to aquatic organisms. We assessed the acute toxicity and sub-lethal effects of pure lubricating oil UNIVIS J 13 in Vibrio fischeri, Daphnia magna, and embryo-larval stages of two amphibian species, Xenopus laevis and wood frog (Rana sylvatica). Aquatic organisms were exposed to a range of percentages (3, 6, 12, 25, 50, 100%) of 1:1 loadings of UNIVIS J 13 water accommodated fractions (WAF). Dose-response relationships were established and lethal loading 50 (LL_{50}) values were determined. The 5-minute LL_{50} in Vibrio fischeri was determined to be 33.4%, and the 48-h LL_{50} in Daphnia magna was determined to be 24%. For Xenopus laevis, there were no significant differences in mortality among treatment groups, but total length, tail length, and snout-vent length were significantly decreased in the 100%, 50%, and 25% WAF treatment groups. For wood frogs, there were no significant differences in mortality nor morphometric endpoints. In order to fully assess the hazard of UNIVIS J13 to a wide range of aquatic organisms, future work aims to evaluate the acute toxicity of UNIVIS J13 WAF in the aquatic plant Lemna minor. However, the toxicity data gained from this study thus far indicates that 1:1 loadings of UNIVIS J13 water accommodated fractions pose de minimis risk to aquatic organisms.

MP321 Health risk assessment of metal induced inhalation exposure to PM2.5 in Korea

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There is a growing interest in the particulate matter, especially PM2.5 (< 2.5μm) in Korea. Annual average PM2.5 concentration from 2006 to 2011 in Seoul was 27μg/m^3 that is higher level than annual standard of WHO (10μg/m^3), USA (15μg/m^3) and even Korea (25μg/m^3). Since PM2.5 is small enough to be inhaled, the continuous high level PM2.5 is a serious problem that threatens the public health. In this study, we evaluated the national level of carcinogenic metals (Cr(IV), As, Ni, Cd) in PM2.5 based on 55 literatures. The exposure parameters for Korean was established to reflect properly the inhalation exposure characteristics of Korean. The lifetime excess cancer risks (LECR) of four metals was calculated following probabilistic approach. The uncertainty and sensitivity of LECR were characterized by Monte Carlo simulation (Crystal ball, Oracle). Of the four metals, the LECR of Cr(IV) and As exceeded the negligible ($< 10^{-6}$) risk but ranged in the acceptable risk (10^{-6}-10^{-4}). The LECR of Ni and Cd were negligible level. Sensitivity analysis showed the metal concentration is a dominant parameter that influence the LECR. Even though the LECR of single metals are not threat, it is necessary to consider the potential risk of metals in terms of the mixture. This study will suggest not only the national risk level of metallic PM2.5 in Korea but also the probabilistic approach to assess the risk of single and mixture compounds.

MP322 Integrated evaluation of air pollution effects: in vitro toxic and genotoxic properties and early biological effects in buccal cells of children

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Air pollution is a global problem. The IARC classified air pollution and particulate matter as carcinogenic to human. Children are a high-risk group in terms of the health effects of air pollution, and early exposure during childhood can increase the risk of developing chronic diseases in adulthood. The MAPEC_LIFE (Monitoring Air Pollution Effects on Children for supporting public health policy) is a project founded by EU Life+ Programme (LIFE12 ENV/IT/000614) which intends to evaluate the associations between air quality and early biological effects in children and propose a model for estimating the global risk of early biological effects due to air pollutants and other factors in children. The study was carried out on 6-8-year-old children living in 5 Italian towns with different levels of air pollutants. Micronucleus frequency was investigated in buccal cells of children. To evaluate child exposure to urban air pollutants, PM0.5 samples were collected for 72h in the school areas using a high-volume air sampler. PM0.5extracts were chemically analyzed for PAH and nitroPAH content and for toxic and genotoxic properties. MTT and NR citotoxicity assays on A549 cells, comet and micronucleus tests on A549 cells, and Ames test on Salmonella strains TA100, TA98, TA98NR and YG1021 were performed. Data on urban air quality during the study period were obtained from the Regional Agency for Environmental Protection. Levels of main pollutants were, as expected, higher in the North of Italy. All PM0.5 extracts showed a mutagenic effect on TA98 and YG1021 strains, suggesting the presence of nitroaromatic compounds, results confirmed by chemical analysis. No genotoxic or oxidative effect of PM0.5 extracts was observed using comet and micronucleus tests. A light cytotoxic activity was detected for all the PM0.5 extracts, except for Brescia sample. Micronucleus frequency in buccal cells of the 1089 recruited children was higher in Brescia (0.06/100 cells) than in any other towns (from 0.03 to 0.05/100 cells). The results showed that, even if all PM0.5 extracts induced mutagenic effect on bacterial cells, they had no genotoxic and only light toxic effects on human cells. Results about micronucleus frequency in children suggested that, in addition to air pollution exposure, some other factors, related to lifestyle or further exposures, may influence micronucleus frequency and cellular response to air pollutants.

MP323 Large Scale Habitat Creation and the Risk Management of the Everglade Snail Kite (Rostrhamus sociabilis); Copper Bioaccumulation

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As part of the ongoing Everglades restoration, former agricultural lands are commonly converted into aquatic and semi-aquatic habitats for water quality improvement and large-scale habitat creation. The conversion of former agricultural areas into aquatic and semi-aquatic habitats creates the potential for mobilization of agrochemicals applied during the active farming of the land. Of particular concern in south Florida is the accumulation of copper, which is widely used as a fungicide in citrus agriculture, into the Florida apple snail. The apple snail is the primary food source for the federally endangered Everglade snail kite and has been shown to rapidly accumulate copper to concentrations of potential concern for feeding snail kites. The SFWMD has completed a series of bioaccumulation tests intended to provide a set of soil/sediment benchmarks that are protective of the Everglade snail kite that can be used as remediation goals during water resource project construction. Initial studies, completed in 2014, exposed juvenile apple snails to sandy soils collected from various locations across south Florida containing copper concentrations between 35 and 120 mg/kg in a series of small (8 ft. by 15 ft.) mesocosm cells. As part of the initial study, a set of threshold tissue concentrations (TSCs) protective of the snail kite were also calculated. The results indicated that concentrations of copper in sandy soils up to 120 mg/kg may be acceptable to leave in place prior to flooding of former citrus groves. Subsequent studies have assessed the difference in copper desorption and bioaccumulation between sandy soils and soils containing much higher levels of organic carbon and have proposed higher benchmarks in areas with highly organic soils (i.e. > 20% OC). The SFWMD is currently conducting a third phase of studies aimed at reducing uncertainties in the original studies and providing additional, more focused data by assessing bioaccumulation within functional water resource project areas that contain sediment copper concentrations at or near the proposed soil benchmarks. The results of the studies will be used by the Interagency Copper Science Review Panel (ICRSP) stakeholders to develop better risk management strategies and balance risks to the Everglade snail kite with the benefits of large-scale habitat creation as part of the restoration of the Everglades.

MP324 National Endangered Species Assessment of Malathion: Problem Formulation and Step 1 Review

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National endangered species assessments (NESA) are currently underway for three case study pesticides (e.g. chlorpyrifos, malathion, diazinon). The Environmental Protection Agency (EPA) and the Fish and Wildlife Service (FWS) (the Agencies) have released their preliminary biological evaluations (BE) for the three organophosphates and the 60-day comment period has past. The BEs have been informative and provided insight into how the Agencies are approaching the malathion evaluation. We have been conducting an aquatic and terrestrial NESA building upon our refined ecological risk assessment for malathion re-registration under FIFRA. We have completed a conservative screening-level risk assessment (Step 1) and are well into the refined assessment (Step 2). We are conducting these analyses for entire receptor groups, focal species as surrogates, and where appropriate specific listed species. Selected approaches are described and key considerations in data quality and multiple lines of evidence are discussed to provide support and context to the risk conclusions. This presentation provides an update to this process and highlights some of the differences in our approach relative to the Agencies BEs.

MP325 Perfluorinated compounds in surface water and sediments of Diep and Plankenburg rivers in Western Cape South Africa

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In this study, seasonal variation and distribution of nine perfluorinated compounds (PFCs) in surface water and sediments of Diep and Plankenburg Rivers in Western Cape, South Africa were assessed. Nine PFCs were extracted from water and sediments samples using solid phase extraction (SPE). Thereafter, the concentrations of the PFCs were determined using Ultra performance liquid chromatography coupled with quadrupole time of flight (UPLC/QTOF). Levels of PFCs in sediments were higher than in water samples from corresponding sampling points. Priority compounds (PFOS and PFOA) were detected in all samples, with concentrations ranging between (71.05 -1090.92 ng/L) for PFOS, and (6.67 – 2144 ng/L) for PFOA in surface water. The levels in sediment ranged between 0.50 and 248.14 ng/g dw, for PFOS and 9.70 and 156.8 ng/g dw, for PFOA. The maximum values for the partitioning/distribution coefficient (log K_{OC}) were 1.311 and 1.316 respectively for PFOS and PFOA. Elevated levels of PFCs were detected in surface water and sediment samples in summer months relative to levels detected during winter. Levels of PFOS and PFOA in surface water were higher than recommended threshold limits (200 and 400 ng/L) for PFOS and PFOA respectively for surface water. These values may be attributed to the presence of informal settlements and industrial facilities in the vicinity of the sampling locations. Anthropogenic activities seem to be a major source of input of PFCs into the aquatic ecosystem, although this present study could not verify this. Keyword: PFOS, PFOA, Surface-water, Sediment, Season, UP/LC-QTOF.

MP326 Potential Human Toxicity of Petroleum Biodegradation Metabolites in Groundwater Samples from Fuel Release Sites

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Petroleum hydrocarbons biodegrade in the environment and are converted to polar (non-hydrocarbon) metabolites. These metabolites are extracted from groundwater samples and quantified as Total Petroleum Hydrocarbons (TPH) unless a silica gel cleanup (SGC) step is applied to the sample extract prior to the TPH analysis. The polar metabolites can be separated from the non-polar hydrocarbons by incorporating the SGC step; however, some regulatory agencies are hesitant to adopt this method, citing the unknown nature and toxicity of these complex mixtures. Non-targeted GCxGC-MS analyses of groundwater samples from historic fuel release sites have tentatively identified over 1700 unique polar metabolites representing many distinct structural classes of chemicals including acids/esters, alcohols, phenols, ketones, and aldehydes. The potential chronic toxicity of these metabolites to human receptors has been investigated. Evaluation of potential chronic human toxicity of the metabolites was initially assessed by reviewing available agency-derived Reference Doses (RfDs) for individual potential metabolites, and then developing an RfD-based toxicity ranking system for each structural class of potential metabolites. This ranking system was applied to all metabolites identified in each groundwater sample. Results show that the vast majority of the metabolites are in structural classes that are ranked "Low" toxicity to humans (RfDs≥0.1 mg/kg/day). In addition, upgradient (representing local background conditions) and downgradient groundwater samples were collected from representative biodegrading fuel release sites and submitted to a commercial laboratory to screen for genotoxicity using the in vitro gamma-H2AX assay, and for estrogenic effects using the in vitro Estrogen Receptor Transcriptional Activation (ERTA) assay. Results of these screening assays indicate that the metabolite mixtures do not appear to have the potential to cause significant genotoxic or estrogenic effects. Overall, the complex mixtures of polar metabolites identified in groundwater are unlikely to present a significant risk to human health.

MP327 Regional distribution of ecological risks of pesticides in Japan – Integrated analysis of environmental model and species sensitivity distribution

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We analyzed regional distribution of ecological risks of 67 pesticides (commonly used in Japanese paddy fields) in 350 sites of Japanese river water. Region-specific environmental models and species sensitivity distribution (SSD) were integrated to quantify ecological risks in each site. Environmental model used in this study consists of environmental scenario (property of river basin) and environmental dynamics from paddy field to river. The predicted environmental concentrations (PEC) in river water was calculated considering pesticide mass discharge through surface runoff and seepage using physico-chemical parameters (soil adsorption constant and half-life in water) and paddy field lysimeter test data. To predict region-specific PEC, the important region-specific parameters of environmental scenario, which are river flow, paddy rice cropped area, and pesticide usage ratio in the basin, were organized at 350 river sites in Japan. The calculated region-specific PECs were validated by comparing with measured concentrations in a river. Differences between measured and predicted concentrations were within 10-folds for all but one pesticide. The SSDs of the 67 pesticides were also analyzed based on acute toxicity data. To do so, ecotoxicity database was constructed by collecting acute toxicity data for freshwater organisms. The magnitude of ecological risk was quantified as the index of potentially affected fraction (PAF) by jointing SSD and the regional distribution of PEC. Finally, we developed the database of PECs and PAFs and the Google map based visualization tool of the data at the 350 sites in Japan. Several techniques for filling the gaps of fate and effect data were also developed.

MP328 Ring test to improve the OECD 306 marine biodegradation screening test

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Recent ECETOC workshops have recommended a series of modifications and enhancements to existing OECD biodegradation screening tests, to deliver more robust methods for assessing persistence. Specific enhancements investigated included increased test durations and investigating the impact of biomass density and diversity on the probability of observing biodegradation. These proposed steps were designed to minimise the high variability and poor reliability previously reported in OECD biodegradation screening tests, such as the OECD 306 marine biodegradation test, whilst increasing the ecological relevance of such studies. The Cefic-LRi funded Eco11 project investigated and validated these enhancements. As a follow up in 2015, the UK Centre for Environment, Fisheries and Aquaculture Science (Cefas) hosted an international workshop on the OECD 306 test in Lowestoft. One of the outcomes from the workshop was an agreement between the chemical industry, regulators and academia that there was too much variability in existing marine biodegradation screening tests and that there was a need to develop a revised method and initiate a ring test of an improved OECD 306 test. This ring test project commenced in January 2016, funded by Cefic-LRi. Laboratories from North America, Japan and Europe, regulators, industry and academia are involved. The ring test aims to validate the use of increased biomass densities and an extended test duration to obtain a more environmentally relevant OECD 306 screening test. Seawater concentration by tangential flow filtration will increase the likelihood of including specific degraders, whilst longer test durations will allow us to investigate previously reported long lag phases in marine systems. A summary of the ring test protocol together with preliminary test results and the status of the experimental work will be presented.

MP329 Risk Reduction Assessment as a means of Quantifying Benefits of a Potential Dredged Material Containment Facility

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As part of due diligence for planning a potential Dredged Material Containment Facility (DMCF), the Maryland Port Administration (MPA) considered Coke Point, a peninsula on the Patapsco River in Baltimore Harbor, Maryland. Preliminary concepts for the DMCF included configurations that would utilize or cover near-shore areas within the Patapsco River. The Patapsco River is a highly urbanized estuary subject to chemical inputs from a wide variety of sources, including legacy pollution, urban runoff, industrial discharges, and known disposal sites. Planning studies found that sediments contain elevated concentrations of metals, polycyclic aromatic hydrocarbons, and polychlorinated biphenyls. One of the potential benefits of a DMCF which extends into the river is that it may cover over areas of elevated concentrations and thus reduce risks to people, wildlife, fish, and crabs from legacy pollutants. Therefore, risk assessment and management studies were performed to quantify the beneficial risk reduction associated with the DMCF to help inform project planning and coordination. A human health and ecological risk assessment was performed for the area offshore of Coke Point that quantified risks from chemicals detected in sediment, water, fish, and crabs. Human health risk assessment focused on recreational and commercial uses, and drew from field studies of bioaccumulation into important fisheries species. Ecological risk assessment focused on the potential for impacts to fish, crabs, and wildlife. Special focus was given to differentiating risks due to chemicals associated with possible sources at Coke Point from risks associated with more widespread urban inputs. Risk assessment results were used to map areas producing the highest risk. Spatial analyses were used to quantify the potential risk-reduction benefits of the project and the possible need for risk management actions. Risk reduction was predicted for numerous potential DMCF design concepts, and potential benefits were incorporated into feasibility studies for the project.

MP330 RiskChallenge: A simulation of the risk assessment process for humans and ecological receptors

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RiskChallenge is an innovative simulation that demonstrates the principles and practices of human and ecological risk assessment and the challenges of resource-limited, multi-stakeholder decision-making. It gives participants an opportunity to apply their risk assessment skills to realistic, hypothetical scenarios, such as those involving contaminated sites and new chemicals. The steps in the risk simulation follow, but do not mimic exactly, those which might be present in various national regulatory programs in the US or elsewhere. The studies and data that participants can purchase for the risk assessment are based the experience of the simulation developers, or from publicly available reports. As a member of a team of stakeholders, participants formulate the problem, assess the exposure and effects data, characterize the risk(s), and make a risk management recommendation. Finite funding and time are given to gather the data needed for the analysis. After the simulation, management recommendations, approaches, frustrations, and uncertainties are shared with other teams. The simulation provides valuable, hands-on experience in the human health and ecological risk assessment process. There are no winners or losers. The goal is simply to increase interest and understanding in risk assessment that will facilitate its use in environmental decision-making across public and private sectors. The simulation is designed to be balanced, objective, and realistic, so it is valuable and entertaining to virtually all stakeholders. While geared toward individuals with environmental backgrounds, virtually anyone can participate. Once tested and validated, this simulation will be donated to SETAC for scientific outreach and education.

MP331 Storm wave modeling (sudestada) – coastal flood risk

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The metropolitan area of Buenos Aires is the most densely populated area of the Argentina republic, a large area of this region is below 5m elevation, vulnerable to flooding during storm surges passage (sudestadas) considered. Coastal areas and cities located in them, are threatened by a wide variety of climate-related hazards. The Argentina coast of Río de la Plata and in particular, the metropolitan area of Buenos Aires, do not escape this situation, becoming increasingly vulnerable because of climate change. The overall objective of this work is the mathematical modeling of a wave of storm using the open source model Delft3d in the Río de la Plata, to assess the risk of coastal flooding. For execution digitized nautical charts for the depths of the study area, which then interpolated triangularly were assigned to the nodes of the grid computing, built by the RFGRID And QuickIN model modules. And also from the tidal constituents obtained for the study site, the model was calibrated with data from the National Hydrographic Service tide predicted for different coastal points of interest. The adjusted model calibration and was then used to simulate a remarkable sudestada occurred in the month of May 2000, using season winds aeroparque city of Buenos Aires and waves on the ocean edge of deep water obtained from the database IFREMER for that date. It was obtained from the modeling, the levels and wave height along stations located control over the Argentina coast. It is observed that the amplitude of the wave is amplified as it propagates storm into the Río de la Plata, due to the decrease in width and depth. The peak levels for the climatic conditions were used to map flood irrigation on a site of interest. The methodology is applicable to future scenarios within the context of climate change.

MP332 Synergistic Induction of Metal-Responsive and Oxidative Stress Gene Biomarkers in Placental JEG-3 Cells by environmental Arsenic & Cadmium Mixtures

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Exposure to elevated levels of the toxic metals inorganic arsenic (iAs) and cadmium (Cd) represents a major global health problem. These metals often occur as mixtures in the environment, creating the potential for interactive or synergistic biological effects different from those observed in single exposure conditions. In the present study, environmental mixtures collected using a passive sampling device from two waste sites in China, and identical mixtures prepared in the lab were tested for toxicogenomic response in placental JEG-3 cells. These cells serve as a model for evaluating cellular responses to exposures during pregnancy. One of the mixtures was predominated by iAs and one by Cd. The gene biomarkers heme oxygenase 1 (HO-1) andmetallothioneinisoforms (MT1F and MT1G) previously shown to be preferentially induced by exposure to either iAs or Cd, and metal transporter genes aquaporin-9 AQP9 and ATPase, beta polypeptide ATP7B were measured in order to evaluate the effects from the metals mixtures using dose and time course experiments. There was a significant increase in the mRNA expression levels of HO-1, MT1F and MT1G in mixture-treated cells compared to the iAs or Cd only-treated cells. Notably, the genomic responses were observed at concentrations significantly lower than levels found at the environmental collection sites. These data demonstrate that metal mixtures increase the expression of gene biomarkers in placental JEG-3 cells in a synergistic manner. Taken together, the data suggest that toxic metals that co-occur may induce detrimental health effects that are currently underestimated when analyzed as single metals.

MP333 Utilization of CBEDMS to dynamically evaluate contribution to receptor relative risk to support the allocation of remediation costs

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Relative risk ratios were calculated for constituents of concern (COCs) to apportion remedial costs for potentially responsible party (PRP) to address unacceptable risks associated with non-aqueous phase liquid (NAPL) contaminated sediments. The ratios estimate the potential risk to human and ecological health based on source-specific COCs including polynuclear aromatic hydrocarbons (PAHs), copper, lead, and polychlorinated biphenyls (PCBs). Apportionment was also based on the estimated COC mass, the volumes of affected sediment, and the spatial distribution of PRP-specific COCs. The goals identified in the Feasibility Study included reduction of: PAH-specific cancer risks during recreational activities; PCB exposure to fish and shellfish; benthic ecological risk from exposure to COCs; and, risks to herbivorous birds from exposure to dietary PAHs. Approximately 900,000 records of historical data were compiled from sources including portable document format (PDF) and electronic tables and databases to a cloud-based enterprise database management system (CBEDMS). Records included spatial, analytical, and lithological information; human and ecological toxicity values; and, molecular weights. Human and ecological COC- and location-specific relative risk ratios for multiple sediment lithologies were calculated using COC concentrations and site-specific preliminary remediation goals (PRGs). Integrated with a geographic information system (GIS), the CBEDMS generated uniform and reproducible total results for multiple PAH and PCB datasets (e.g., individual, alkylated, and total PAHs, individual and total PCBs); determined lithologic interfaces for estimation of COC-specific relative risks for vertical profiling of impacts; estimated mass of each COC by location; and calculated risk-driver-specific sediment volumes to be remediated. The CBEDMS allowed for dynamic evaluation of the spatial distribution of COCs and NAPL in sediments, relative risks to human and ecological health, and masses of COCs and volumes of sediment for each risk driver to inform the risk manager in an iterative approach. This enabled the apportionment of remedial costs by identifying PRP contribution to unacceptable levels of risks. This methodology supported a scalable, flexible, and defensible position in the allocation of remedial costs and can be applied to remediation sites from small, multiple party projects to complex Superfund projects and natural resource damage assessments (NRDA).

MP334 Whole Stream Metabolism in Agriculture Drainage Systems

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Agricultural drainage systems influence stream metabolism and productivity by channeling nutrients and other resources from terrestrial ecosystems into aquatic systems. Monitoring ecosystem function in smaller catchments may be more cost effective and practical than measuring the whole ecosystem. This study investigated stream metabolism within replicated experimental agricultural drainage systems during a simulated storm runoff event. Eight vegetated drainage ditches of two types (conventional and controlled with weirs) with contrasting vegetation management strategies have been studied. Four vegetated ditches (two of each type) were mowed with clippings remained in the system, while vegetation in the remaining four ditches was left unmanaged. Results indicated ditches with weirs had significantly ($p < 0.001$) higher gross primary production (GPP) (2.83 ± 0.31 g O_2 m^{-2} d^{-1}) compared with conventional ditches with no weirs (0.60 ± 0.23 g O_2 m^{-2} d^{-1}). The presence of additional vegetation in this system may have contributed to its higher GPP, rather than the presence of autotrophic biomass (algae), because mean chlorophyll a was comparable between ditch treatments. This demonstrates the ability of weirs to play a role in enhancing soil inundation which supports denser autotroph biomass. Community respiration (CR) was significantly ($p = 0.04$) higher in mowed ditches ($- 0.98 \pm 0.39$ g O_2 m^{-2} d^{-1}) than in unmowed ditches ($- 0.20 \pm 0.06$ g O_2 m^{-2} d^{-1}). The higher CR could be attributed to vegetation clippings left in ditches after mowing, which may have increased available organic matter to the system, thus fueling additional heterotrophic microbial respiration. Overall, all ditches were net autotrophic, having positive net-ecosystem production (NEP), and a ratio of GPP: CR >1.0. This study is among the first to demonstrate that agricultural drainage ditches are good representation of streams draining agricultural catchments in assessing whole stream metabolism and function.

Alternative Approaches to Animal Testing for Ecotoxicity Assessments

TP001 A mass spectrometry based metabolomics study of fluoxetine exposure in developing zebrafish embryos

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Aquatic organisms are continuously exposed to a host of pharmaceutically active compounds (PhACs) in the natural water reservoirs leading to inadvertent effects. It is crucial to detect the induced toxicological insult in the early stages, despite there being no morphological changes. Detection of early stage disruptions at the molecular level and profiling of differential metabolites can aid risk assessment endeavours. Fluoxetine is one such PhAC which has potential endocrine disrupting properties and has been detected in aquatic environment worldwide including in Singapore. In the present study in vitro toxicity experiments using embryonic zebrafish were conducted to assess metabolomic changes associated with exposure to a cascade of fluoxetine concentrations. An untargeted metabolomics approach was adopted to furnish the whole body tissue metabolites of zebrafish embryo. The exposure commenced at 2hours post fertilization (hpf) and continued until 96hpf. Larvae were then snap frozen to quench metabolism and arrest enzymatic activity, followed by extraction using a solvent mixture of acetonitrile: isopropanol: water (3:3:2; v/v/v) . The extracted metabolites were subsequently analysed using mass spectrometry (GC-MS). A total of 35 metabolites were positively identified against the Fiehn library database. Multivariate analysis tools (PCA, OPLS-DA) were used to identify metabolites that were differentially regulated with increasing fluoxetine concentration. Hierarchical clustering analysis was applied to the mined data and metabolite classes such as amino acids and lipids were found to be perturbed. Integration of multivariate and univariate analysis revealed significant dysregulation of 11 metabolites. Impediment of the energy and amino acid metabolism could have serious implications for normal and healthy embryo development.

TP002 A mass spectrometry based metabolomics study of triclosan exposure in developing zebrafish embryos

J. Fu, National Univ of Singapore; B.C. Kelly, Barry C Kelly / Civil Environmental Engineering

Triclosan (TCS), used as an antibacterial and antifungal agent, is ubiquitously detected in the natural environment. Increasing studies provided strong evidence of its potential for acting as an endocrine-disrupting chemical (EDC), involving both estrogenic and antiestrogenic effects as reported. It is vital to detect its toxicological impact in the early stage organisms, in terms of molecular level and metabolites profiling, despite no morphological effects was detected when organisms were exposed to TCS in environmental-related concentration. In the present study, in vitro toxicity experiments using zebrafish embryos were conducted to assess metabolomics changes with exposure to a wide range of TCS concentration. Exposure is to be carried out at 2 hpf and terminated at 96 hpf. Larvae were then snap frozen, followed by extraction using a solvent mixture of acetonitrile: isopropanol: water (3:3:2; v/v/v). Subsequently, samples were analyzed with gas chromatography mass spectrometry (GC-MS) to identify the metabolites based on Fiehn library database. As a result, a total of 35 metabolites were positively identified. Multivariate analysis (PCA, OPLS-DA) and univariate analysis (One-way ANOVA) were employed to identify significant dysregulation in metabolites and 11 metabolites showed significant differences due to TCS exposure. This outcome may suggest the biological mechanisms have been effected, including impaired energy production, lipid metabolism and increasing oxidative stress.

TP003 Comparison of human and fish estrogen receptor ligand binding domain-mediated response to estrogenic compounds

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Endocrine disrupting chemicals (EDCs) are compounds that perturb hormonally regulated processes. Xenoestrogens, or estrogen mimics, are a class of EDC that have the potential to cause reproductive dysfunction; they mediate their effects by binding estrogen receptors (ER), causing changes in transcriptional activation. These chemicals enter the aquatic environment through land run-off and water treatment effluents, making their effects on fish populations a concern. However, current in vitro screening assays for xenoestrogens are commonly based on human estrogen receptors, which differ from teleost fish ERs in their amino acid sequences, number of subtypes, and subtype functions. These differences suggest that current assays may not accurately indicate the potential risk posed to fish. In this study, we focus on the role of the ligand-binding domain (LBD) in the ER-mediated response to estrogenic compounds. We utilize transactivation assays to compare ER LBD sensitivities between human and teleost ER subtypes. The hinge (D), LBD (E), and N-terminus (F) region of each ER subtype from human (hERα-def and hERβ-def), zebrafish (zERα-def, zERβ1-def, and zERβ2-def), and rainbow trout (rtERα-def, rtERβ1-def, and rtERβ2-def) are expressed as fusion proteins with a GAL4 DNA binding domain. GAL4-ERdef fusion proteins are used in transactivation assays, conducted in HEK293 cells, to compare the ER LBD-mediated response to a selection of natural and synthetic estrogenic compounds. Focusing on the LBD region of the ER is a targeted approach intended to pin-point differences in ER-mediated responses between species. Overall, this study provides insight regarding the use of fish-based in vitro assays as a means to assess the potential risk of EDCs to aquatic organisms.

TP004 Making the fathead minnow fish embryo toxicity test feasible: Spawning strategies to optimize embryo production

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With the passage of REACH legislation, and its strong push for using alternative toxicity testing methods that improve animal welfare, there has been interest in expanding the fish embryo toxicity (FET) test. In the US, efforts to modify the existing FET test protocol have paved the way for a fathead minnow FET test, the teleost model species typically used for acute toxicity assessments in the US. Previous studies have shown that the fathead minnow FET is similar in its predictive ability to the larval growth and survival test indicating that the FET test may be a viable replacement. However, procuring eggs at an early enough developmental stage (≤32 cell) and in sufficient quantity (>170 embryos/test replicate) to initiate a FET test has been a challenge. For the fathead minnow FET test to be a viable toxicity testing method, a spawning strategy that allows for adequate egg production must be developed. With this in mind, we sought to determine which male:female spawning ratios would optimize egg production and also examined the impact of collection time on the number of ≤32 cell embryos harvested. Specifically, this study compared breeding ratios (1m:4f, 1m:8f, 2m:4f, 2m:8f) and egg collection times (2, 4, and 8h after "sunrise") to determine which combination produced the largest number of ≤32 cell stage embryos. Initial results suggest that breeding colonies containing a single male optimize total egg production and that spawning windows of ≤2h allow for the collection of appropriately staged embryos. Implementation of the spawning methods identified in this study will allow fathead minnow embryo FET tests to be a more feasible option for chemical and effluent acute toxicity assessments. Further studies looking into breeding structure availability, light cycles, and alternative ratios could assist with perfecting breeding methods for the purpose of the fathead minnow FET test.

TP005 Use of surface mucus as a biomarker for endocrine disruption in an estuarine fish

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Reproductive investment and endocrine health are normally assessed using assays which typically rely on blood collection, which is often lethal to small fishes. Surface mucus collection offers a less invasive methodology to determine concentration of 11-ketotestosterone (11-KT) and vitellogenin (VTG). This project seeks to (1) validate and confirm the use of mucal swabs as an effective and non-lethal alternative to determine concentrations of 11-KT and VTG of Fundulus grandis through comparative assays of blood plasma, and (2) use this minimally invasive method to evaluate potential endocrine disruptions in F. grandis undergoing chronic exposure to a water accommodated fraction (WAF) of crude oil, and (3) assess potential differences in endocrine disruption at the population level based on exposure history. Mucus and plasma samples were collected from male and female F. grandis. Both 11-KT and VTG showed significant positive relationships between their concentrations in the plasma and mucus. Four different populations of F. grandis with different exposure histories were exposed to WAF for 60 days, and post-WAF conditions for 40 days. Assessment of mucosal 11-KT concentrations showed that endocrine disruption was observed in many populations throughout time with a severe decline 40 days post-WAF. Changes in concentrations of 11-KT and VTG where significant with the linear relationship of time, treatment and population. Exposure history also had a significant effect on 11-KT concentrations; exposed reference site males had a decrease in 11-KT concentrations, while concentrations of 11-KT of exposed males from contaminated sites increase. Exposed females showed near null concentrations of mucosal VTG when exposed to WAF regardless of exposure history. These data demonstrate the utility of mucus sampling as a less invasive tool to assess endocrine disruption in fish exposed to polyaromatic hydrocarbons in laboratory and field settings.

TP006 Development of a fish liver microtissue model to characterize the toxicity of aromatic hydrocarbons and nanoparticle-based dispersants

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Engineered nanoparticles can assemble at water-oil interfaces to stabilize oil droplets into an emulsion, and are under development for use as dispersants following oil spills. This project focuses on the potential impacts of environmental exposures to these nanoparticles on aquatic organisms. Using a fish liver cell line, PLHC-1, in a three-dimensional (3D), scaffold-free microtissue model, we are examining the environmental impacts of co-exposure to polycyclic aromatic hydrocarbons and surface-engineered carbon black as a model nanoparticle dispersant. 3D cultures can provide the benefits of tissue-like cell-cell interactions and the opportunity for longer term cultures, but also present challenges associated with imaging, maintaining viability, and adapting two dimensional assays to 3D systems. For this novel fish liver microtissue model, assays to determine the toxicity of aromatic hydrocarbons and nanoparticles, including biomarkers of stress and xenobiotic metabolism, were optimized in monolayer and then adapted for use in 3D with toxicants. To characterize the fish liver spheroids, changes in histology, differentiation, and response to toxicants were measured during long term cultures of spheroids. This model is applicable for testing real-world contaminant mixtures, and is being developed as a sensitive screening assay for low-level chronic or cumulative exposures. This research is supported by NIEHS Training Grant T32 ES07272, NIEHS Superfund Research Program P42 ES013660, the Institute at Brown for Environment & Society, and the generous support of Donna McGraw Weiss '89 and Jason Weiss.

TP007 Development of an In Vitro Chicken Gonad and Liver Slice Culture to Study the Effects of Endocrine Disrupting Chemicals

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Exposure to Endocrine Disrupting Chemicals (EDCs) can have adverse effects on the hypothalamic-pituitary-gonadal-liver (HPGL) axis. Disruptions in this pathway can cause changes in reproductive physiology, neuroendocrinology and metabolism leading to population level impacts. Field- and whole-organismal studies have shown that EDCs adversely affect avian species such as chickens, quails and bald eagles. While offering compelling evidence of harm, organismal studies involve sacrificing a large number of animals, are time consuming and expensive. In recent years there has been great interest in developing in vitro systems to study biologically relevant disturbances in interconnected pathways composed of complex biochemical interactions and small molecules that control communication between cells. Among the various in vitro approaches being explored, tissue explants show great promise. For example, explants from neonatal rats and embryonic chicken retina had superior histology, showed remarkable retention of architecture and were more similar to freshly isolated tissue than 2-D cell culture. Given the promise of tissue explants as an alternative in vitro model, the objective of this study is to establish an in vitro slice culture of gonadal and liver tissue from day 19 chicken embryos. Optimal methods included slicing gonads and liver in chilled phosphate buffer and culturing for 24 hours at 37°C and 5% CO_2. Gonads were treated with pregnant mare serum gonadotropin (PMSG – 0, 2.5, 5, 10IU), and mRNA expression of genes involved in steroid biosynthesis viz. 17β-hydroxysteroid dehydrogenase (17β-HSD) and aromatase (CYP19A1) were analyzed. Liver slices were treated with 17β-Estradiol (E2 – 0, 0.1, 1.0, 10, 100, 1000nM) and vitellogenin (VTGII) mRNA expression was analyzed to assess functionality of the cultures. Gonads dosed with PMSG did not show any changes in 17β-HSD and CYP19A1 expression. Liver slices dosed with E2 at 0.1 and 1.0nM showed a 5 ± 1.7- and 8 ± 2.1-fold increase in VTGII expression. However this dose response did not extend through to the 10, 100 and 1000nM treatments. Studies to optimize the assays are ongoing following which the cultures will be dosed with EDCs of interest. Understanding the effects of contaminants on the HPG-L axis is important in determining the detrimental effects that EDCs have on humans and wildlife at organismal levels, and this in vitro model could serve as a valuable screening tool to study these interactions.

TP008 Characterization of gene expression in lake trout, northern pike and rainbow trout following exposure to EE2, fluoxetine and HBCD in vitro

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Large numbers of chemicals are discharged into aquatic ecosystems as a result of human activities. While some of these compounds have been widely studied and adverse effects on fishes have been identified, there is an increasing number of emerging contaminants (ECs), including pharmaceuticals and personal care products (PPCPs) or brominated flame retardants (BFRs), for which little or no toxicity data regarding aquatic organisms is available. Many of these ECs, such as 17α-ethynylestradiol (EE2), a potent estrogen agonist used in oral contraceptives, fluoxetine, a common antidepressant, and hexabromocyclododecane (HBCD) a widely used BFR, may pose significant risks to aquatic ecosystems due to their prevalence in the environment.Specifically, large quantities of these chemicals have been identified in municipal wastewater effluents (MWWEs). Widespread use of ECs has raised concerns regarding their possible risks to the environment, particularly to native species of cultural, recreational and commercial importance to Canadians, including lake trout (Salvelinus namaycush) and northern pike (Esox lucius). While extensive data are available on model laboratory species, such as rainbow trout (Oncorhynchus mykiss),little is known of the effects of these ECs to species in northern ecosystems, and as such, there are approaches needed that allow to assess potential effects to such species. However, there is increasing concern with regard to live animal testing, particularly with regard to endangered or wild species, and therefore, alternative testing methods, such as in vitro tissue explant assays, are needed. Following an in vitro tissue explant assay in which lake trout, northern pike and rainbow trout livers were exposed to serial concentrations of EE2, fluoxetine or HBCD, transcript abundance of select genes was measured in these species and a species-specific response was characterized. With exposure to EE2, fluoxetine or HBCD, no response was observed in expression of genes related to endocrine disruption or oxidative stress in any species tested and, therefore, these species are relatively more tolerant to these chemicals than other species previously tested.

TP009 Sampling Season, a Factor Influencing Prevalence of Gonadal Intersex and Molecular Biomarkers in Smallmouth Bass

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Over the past decade, studies have shown that exposure to estrogenic chemicals can cause gonadal intersex in fish. Smallmouth bass (Micropterus dolomieu) males appear to be highly susceptible to developing this condition in the form of testicular oocytes with cases reported in various areas across the U.S. The main objective of this study is to identify molecular biomarkers of gonadal intersex in smallmouth bass. First, we studied the prevalence and severity of gonadal intersex in smallmouth bass sampled from the St. Joseph River, an impacted river in northern Indiana, at several sites, seasons and years. Our results showed variable prevalence and severity of gonadal intersex, ranging from 0% to 100% and score 1 to 4, respectively. Over the different years tested, the prevalence and severity of gonadal intersex was always significantly higher in spring (spawning season) when compared to the prevalence in summer (post-spawning) of the same year. Next, we quantified molecular biomarkers of intersex in smallmouth bass by studying the differential expression of gonadal genes involved in sex differentiation (esr1, esr2, foxl2, vtg, cyp19a, cyp1a, star, lhr and fshr). Hepatic vitellogenin (vtg) transcript levels were significantly higher in males with gonadal intersex compared to normal males, but only in the spring. Finally, we investigated the levels of VTG plasma protein in males with and without gonadal intersex using Western blot analysis. Our results confirmed our earlier findings by showing detectable levels of VTG in males with gonadal intersex that also had high levels of vtg transcript expression. Overall, our results show that the prevalence and intensity of intersex is highest during spring, and that VTG is a successful biomarker of gonadal intersex only during the spawning season.

TP010 Perfusion based Lab-on-a-Chip technology for automated toxicity testing with the marine amphipod Allorchestes compressa

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Amphipods have gained popularity as excellent bioindicators. They are important links in numerous food chains and have localized behavior that can be used to assess sediment toxicity and water quality. Bioassays performed with amphipods, however, largely still use static conditions in large test volumes, require manual manipulation of samples, and employ mortality as the major test criterion, since sublethal tests including behavioural tests are time and labour intensive and can be subject to 'Observer bias'. This work describes design and validation of a miniaturized, continuous perfusion based Lab-on-a-Chip technology for automated sub-lethal behavioural toxicity tests using the native Australian marine amphipod Allorchestes compressa. An automation module with a high-resolution USB camera, user-friendly fluidic interconnects and miniaturized 3D-printed interface was developed. To evaluate performance of the new chip-based system, median lethal concentrations (LC_{50}) of a panel of reference toxicants obtained on this system were compared with those from tests using conventional static protocols, and were not significantly different. Automated behavioral tests were then conducted by perfusing toxicants through the chip-based device to dynamically assess the effect of toxicants on selected locomotory parameters. Results showed that the system was able to detect changes in the swimming behavior of A.compressa at toxicant concentrations that did not induce mortality in test populations. For the majority of chemical stressors tested, behavioral sub-lethal changes occurred early and in a concentration- and exposure time-dependent manner and could be recorded with no observer input. We postulate that integrated Lab-on-a-Chip systems can enable new avenues for "Early Warning" biomonitoring systems that can automate the use of sensitive behavioral indices to rapidly detect presence of toxicants in aquifers.

TP011 Effect of Petroleum Refinery Effluent on Haematological and Biochemical Characteristics of Juvenile African Catfish (Clarias gariepinus)

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Effect of Petroleum refinery effluent on hematological and biochemical characteristics of juvenile African catfish was investigated. Twenty five juvenile African catfish with the average weight of 3.0 kg were subjected to four graded sub-lethal concentrations (0.002, 0.006, 0.01 and 0.016 mg/l) of petroleum refinery effluent and water (control) for fourteen days. A significant reduction ($p< 0.05$) was observed in haemoglobin concentration (Hb), pack cell volume (PCV), red blood cell (RBC) and white blood cell (WBC) counts compared with the control. Reduction in Mean corpuscular volume (MCV) and Mean corpuscular heamoglobin concentration (MCHC) were however not significant ($p>0.05$). Result of the biochemical studies shows significant ($p< 0.05$) increases in the activities of Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) in the samples exposed to petroleum refinery effluent, suggestive of hepatic cellular damage. Protein concentration was significantly ($p< 0.05$) lowered in the samples exposed to the effluent while the reduction in albumin was not significant ($p< 0.05$). This study has shown that alterations in the levels of haematological and biochemical parameters of African catfish can be used as an indication of potential hazards of petroleum refinery effluent on aquatic biota.

TP012 A call for consistency: What is the most appropriate form of euthanasia for fish

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Shell has a global animal testing policy and associated guidelines which are reviewed by an external committee and updated regularly to guarantee 'best practices' for the humane use of animals. In 2014 Shell used roughly 82,000 fish for product or whole effluent toxicity testing, which is the largest category of vertebrate animal testing conducted by or on Shell's behalf. Shell's guidelines include a discussion of the euthanasia of laboratory animals. In the Shell animal testing guidelines, euthanasia means, literally, an easy or good death and in practical terms, this means that the animal should be killed causing minimal pain or distress. The guidelines reference the American Veterinary Medical Association (AVMA) Guidelines on Euthanasia, and the Newcastle Consensus of 2006 on carbon dioxide euthanasia. However, no specific method of euthanasia for fish is recommended in the Shell guidelines. To remain at the forefront of humane fish handling practices, these guidelines and the referenced documents were reviewed with the objective of identifying a method of euthanasia specifically for fish. Two common methods of euthanasia were reviewed: 1) CO_2-saturated water and 2) buffered tricaine methanesulfonate (MS-222). Use of CO_2 with terrestrial animals can cause rapid asphyxiation and the AVMA guidelines supports its use for the humane euthanasia of both terrestrial and aquatic animals, though little information actually exists supporting this position for fish. Further, other organizations such as the Canadian Council for Animal Care (CCAC) strongly advocate against the use of CO_2 for euthanasia of fish. The CCAC presents that the addition of CO_2 to water forms carbonic acid which can alter the pH of the aquatic environment causing undue suffering for the fish. Indeed, the AMVA guidelines warn that CO_2 might cause pain due to formation of carbonic acid on the respiratory and ocular membrane of terrestrial animals, though they do not extend this consideration to an aquatic environment. The use of the anesthetic, MS-222, was also reviewed as it is currently a prominent method of euthanasia. Both the AVMA and CCAC guidelines suggest the use of an overdose of MS-222 for humane euthanasia, though recent studies suggest it might be aversive to fish. Following an extensive review of best practices and literature representing state of the science, we proposed the discontinued use of CO_2 and the use of MS-222 as the prominent method of euthanasia for Shell testing.

TP013 Use of the RT-gill W1 Cell Assay for Predicting Fish Acute Toxicity – In vitro to In vivo Comparison for Fragrance Molecules

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Considerable effort has been invested in recent years in the development and validation of testing methods aimed at fulfilling the principles of the 3Rs (Reduction, Refinement and Replacement of animal tests), while maintaining a high degree of scientific reliability. Regulatory relevance is also a major consideration in the development, validation and implementation of alternative testing techniques. The OECD Test Guideline 236, Fish Embryo Acute Toxicity Test, was adopted in 2013 offering an alternative to the OECD 203. Tanneberger et al. (2013) proposed a further simplification step through the use of a fish gill cell line assay as a means of predicting acute toxicity. This approach has been applied in our research laboratories with the Rainbow Trout gill cell line RTgill-W1 for 40 fragrance molecules, with wide ranging physico-chemical properties, and broadly differing intrinsic toxicity as determined from reliable in vivo studies. The findings of this benchmarking study reveal a strong correlation ($r^2 = 0.93$) between the In vivo LC50 values, based on fish mortality, and the In vitro EC50 values based on cell cytotoxicity for the fragrance molecules tested. Furthermore, the overall extrapolation illustrates a median under-/over-prediction from In vitro EC50 values to In vivo LC50 values of a factor of 1.68 which is well within the variability associated with the OECD 203 test guideline when the effects of inter-species and inter-laboratory variation are accounted for. This assay clearly offers a simple, accurate and reliable alternative to In vivo fish acute toxicity testing for chemicals presumably acting mainly by narcotic mode of action. This study provides validation of the assay on an independent set of chemicals not tested before and indicates that fragrance chemistry clearly is within the applicability domain of the assay. The assay protocol is currently being evaluated in an international inter-laboratory ring test, required as a pre-requisite for adoption as a draft OECD test guideline.

TP014 Construction and curation of a large ecotoxicological dataset for the EcoTTC

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The Ecological Threshold for Toxicological Concern, or ecoTTC, has been proposed as a natural next step to the well-known human safety TTC concept. The ecoTTC is particularly suited for use as an early screening tool in the risk assessment process, in situations where chemical hazard data is poor, or when an appropriate QSAR is unavailable. EcoTTCs are developed using statistical distributions of Predicted No-Observed Effect Concentrations (PNECs) to reflect the breadth and depth of the ecotoxicological dataset beneath. Therefore, the diversity and quality of the underlying dataset is crucial to the future utility of the ecoTTC. A database consisting of approximately 110,000 unique ecotoxicological records has been created based on recent assessments of published data and international chemical management programs. Stepwise data selection strategies, query systems and curation techniques are applied to ensure a transparent, methodical process towards a final dataset on which to found development of ecoTTCs. Reference-sourced toxicity data is associated with physical chemistry data and taxonomic information for the tested chemical. The resulting dataset contains information on 6200 unique CAS numbers for 1900 species in three trophic groups for use in ecoTTC development and associated metadata mining. This presentation does not necessarily reflect the views or the policies of the USEPA or the European Commission.

TP015 Small-Scale Microcosm Assay to Assess the Environmental Fate and Ecotoxicity of Nanomaterials in Aquatic Systems

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With the ever-growing diversity of nanomaterials in commerce, it is impractical to utilize current testing paradigms to evaluate each material for its potential environmental impacts. Given the immediate need to quickly and cost-effectively screen nanomaterials for ecotoxicity, data-rich assays that are rapid, biologically representative, easily implemented, and readily amenable to inter-laboratory standardization are of significant value to the scientific community. The goal of this research was to establish a novel, ecologically relevant, small-scaled freshwater microcosm assay for simulating realistic exposure scenarios and understanding community response to nanomaterials. Microcosms comprised of algae (Chlamydomonas reinhardtii), bacteria (Escherichia coli), predatory invertebrates (Daphnia magna), and developing vertebrates (Danio rerio) were used to evaluate biological and ecological responses to nanomaterial. Two commercially available ZnO NPs were evaluated in each of the single organisms in addition to community exposures. Our results showed that engineered ZnO NPs can elicit differential toxicity among test organisms at 0.01 and 1 mg L^{-1}, and overall, less toxicity was observed for each species when exposed as a community. Next, a series of 70 nm AgNPs [polyethylene glycol (PEG), silica (Si), or aminated silica-coated AgNP (Ami-Si) at 0, 0.1, 1, and 5 mg L^{-1}] were used to investigate the relative influence of surface charge, composition, dissolution and organismal uptake on toxicity. We found that PEG-AgNPs had the highest overall toxicity followed by Si-AgNPs, and lastly Ami-Si-AgNPs. Increasing species complexity in this study also reduced the toxicity to algae and bacteria. Our findings indicate that surface functionalization plays an important role in determining dissolution, uptake and toxicity of AgNPs. Increasing trophic complexity in both experiments decreased organismal susceptibility under the same NP exposure concentrations, likely due to changes in bioavailability. Thus, single-species tests provide conservative estimates of environmental impacts, as exposures may be mitigated in more realistic multi-species scenarios. Rapid testing strategies, such as the nanocosm assay, will enable researchers to meet both current and future testing needs, as well as rapidly improve our understanding of risk from environmentally-relevant exposure to nanomaterials.

TP016 Using D. magna as an alternative to fish toxicity mitigation testing for cationic substances

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Cationic polymers (CPs) are polyelectrolyte chemicals used widely in industrial applications (e.g. water clarification) and as a functional component of various "down-the-drain" household products. CPs generally have a low environmental concern due to the presence of organic carbon (OC) in surface water that greatly reduces their bioavailability, even at low mg/L levels. To better understand CP toxicity under environmental exposure scenarios, USEPA recommends establishing baseline acute fish toxicity in clean water and conducting at least 2 additional acute fish toxicity tests at higher total OC (TOC) levels to evaluate the mitigation potential. Daphnia acute toxicity tests are also required as part of the baseline aquatic ecotoxicity dataset and are a suitable alternative to assess mitigation, fitting easily into established regulatory frameworks. Clean water used for baseline toxicity tests should have TOC < 2 mg/L, but guidelines allow any water, ranging from defined artificial water to natural surface water, as long as general criteria are met. Subtle water chemistry variations within the clean water criteria are believed to have little influence on the toxicity outcome. To better understand variation in clean water studies, we explored the impact of ppb additions of TOC in artificial water with different hardness on CP toxicity. We used CPs of varying molecular weights and cationic charge density in Daphnia acute toxicity tests. Our results indicate that ppb levels of TOC produced strong CP toxicity mitigation. In addition, water hardness was an important co-mitigating parameter for the toxicity of all polymers tested. These results suggest that clean water variations of TOC and water hardness affect baseline CP toxicity and should be considered in environmental exposure scenarios. We recommend measures to enhance the reliability of baseline toxicity tests with cationic polymers while representing an environmentally realistic worst case exposure.

TP017 Are alternative strategies actually faster, cheaper and more efficient than traditional methods?

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Chemical contamination is considered to be one of the planet's greatest threats (~100,000 chemicals are currently registered in commerce). Animal based toxicity testing methods are not feasible with respect to number of animals used, money and time spent. Hence, typically, high risk classes of chemicals undergo rigorous toxicity testing. As a result, either chemicals that are unknowingly highly toxic might be in widespread use or, obtaining regulatory approval for emerging chemicals might get delayed. Seminal reports over the last decade have pushed for a paradigm shift in toxicology, with more emphasis on mechanistic, screening platforms to prioritize chemicals for in vivo testing. Several alternative methods have been developed for toxicological purposes that are professed to be faster, cheaper and use fewer animals than traditional in vivo models. The purpose of this study was to perform a bibliometric search to identify and review papers covering the following five crucial areas when comparing alternativeto in vivo methods, viz. A) cost of testing (toxic* AND alternat* AND economic* AND benefit*), B) time needed for testing ("in vitro" OR screen*) AND ("short-term" OR time OR ((high OR increase*) AND throughput)) AND toxic*, C) number of animals needed ("in vivo" AND toxic* AND "animal testing" AND number*), D) predictability of alternative methods (("in vitro" OR "high throughput" OR alternat*) AND screen* AND "in vivo" AND predict*) and E) regulatory acceptance of data from alternative methods ((alternat* OR "in vitro") AND toxic* AND "risk assessment"). In general, a search for ("in vitro" AND "toxic* AND screen*) found 3,467 publications between 2007 and 2016 as compared to 1,094 between 1997 and 2006. A few empirical examples were found in support of the five areas of inquiry. For example, custom designed qPCR arrays can yield screening level information in ~3 weeks compared to ~6 months ordinarily needed for standard tests. 'omics based technologies are estimated to yield 25-fold cost savings. Regulatory testing (ex. Pulp and Paper Mill) uses animals to test all substances, while the compliance rate is ~97%. Results from screening assays for estrogenic and androgenic activity predicted in vivo responses with high accuracy, however thyroid related effects could not be predicted accurately. However, taken together, there are few studies that have empirically examined the notion that alternative methods are faster, cheaper and more efficient.

TP018 Evaluation of toxic effects of detergent in embryos and adult zebrafish (Danio rerio)

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In ecotoxicological studies, the zebrafish has been used as a test organism for assessing the effects of chemical compounds mainly. In toxicity tests, has been used to embryos of this species because its response comparable to the sensitivity of adult organisms. In Mexico, the use of these organisms is not stipulated in the legislation, despite being an alternative to replace fish bioassays. The aim of this study was to determine the toxic effect of two surfactants: LAS and Triton X and 5 products (Extram, Ace, Ariel, Foca and Roma) in embryos and adult zebrafish Danio rerio to compare their sensitivity. Static bioassays were conducted with a duration of 48 and 96 hours, embryos and adult fishes were exposed to 5 concentrations of toxic (12 replicas), plus a control without toxic. LC_{50} was determined and a comparison of the LC_{50} obtained to test are made to detect if the responses of embryos and adult fish are different. The toxicity of surfactants was (high to low toxicity): TX > LAS. And products: Extram > Ariel > Roma > Ace > Foca. Comparison of LC_{50} indicated no significant differences in the effects of detergents between embryos and adults in tests with Ace, Foca and Roma products. But if observed differences in the response of adults and embryos exposed to surfactant and the detergent Ariel. The embryos were more sensitive to these products. The results indicate that tests with zebrafish embryos are a useful tool in monitoring studies. Because wastewater discharges in the Valley of Mexico contain high concentrations of detergents (0.5-100 ppm) and their final destination is the aquatic systems, for this reason it is important to continue research to detect responses that indicate the possible adverse effect on fish by the action of different the discharges and tensors to prevent irreversible deterioration of the populations in the medium and long term.

TP019 Testing toxicity of graphene oxide in zebrafish embryos

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Due to their unique physiochemical properties, two dimensional graphene oxide (GO)-based materials have attracted considerable attention both in research community and industry. With the more application of this nanomaterial in the production of goods used in our daily life, it is increasingly released into the environment. However, the health risks associated with environmental exposure to GO are largely unknown, particularly with respect to embryogenesis. Zebrafish embryos are an alternative model for the evaluation of developmental toxicity of chemicals during early life stage with the characteristics of small-scale, high throughput and easy observations. In preliminary experiments, 4 hours-post fertilization (hpf) zebrafish were treated with 8 concentrations of GO (water dispersion): 0.1, 0.3, 1, 3, 10, 30, 100 and 300 mg/L. A hole in the chorion was performed with a thin needle to prevent toxicity induction by hypoxia due to graphene adhesion to the chorion. Embryos were analyzed daily until 4 days post-fertilization (dpf) and not toxicity manifestations or lethality were observed at any of the concentrations tested. To guarantee the uptake of graphene, a second experiment in which graphene was injected was carried out. Two concentrations were selected, 3 mg/L, the concentration from which aggregation of graphene was clearly detected in the first experiment and one lower concentration, 1 mg/L. Toxicity manifestations were not detected in this case either. To further ensure that the absence of effect is not due to the absence of GO uptake, a smaller particle size preparation of GO will be also tested. Additional behavior test will be also performed, besides the morphology evaluation, to detect possible toxicities.

Lab Data, Hazard, Risk and Regulation of Endocrine Active Chemicals – So What? The Big Picture from Little Pieces

TP020 Neonatal exposure to bisphenol analogues disrupted genital development in male mice

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The public concern and governmental regulations on bisphenol A (BPA) have stimulated the development and production of alternative substances to replace BPA in a myriad of applications. A total of 16 bisphenol analogues that are structurally similar to BPA have already been used in the manufacture of polycarbondate plastics and epoxy resins, as well as other industrial applications. Given the endocrine disrupting activities of BPA and potentially other analogues, the present study investigated and compared the effects of neonatal exposure to BPA, BPB, BPE, and BPS on the genital development in male mice. Pups were injected subcutaneously on the right shoulder in the mornings of days P0.5, P2, P4, and P6, resulting a dose level of 50 µg/g body weight/day. Mice were sacrificed at the weaning day (P21) and were evaluated for four parameters, including anogenital distance, glans penis length, glans penis morphology, and testis weight. The results demonstrated that BPA, BPB, or BPE significantly shortened glans penis length, while BPS didn't. No difference in penis length was observed between any two treatments of BPA, BPB, and BPE. Testis weight and anogenital distance were also significantly affected by all four bisphenols. Testis weights in BPA treatments were significantly lower than those in BPE or BPS groups, but no difference was observed between BPA and BPB treatments. These results suggested that bisphenol analogues other than BPA may possess similar endocrine disrupting effects compared to BPA. This raised the concern if some of these analogues can be used as safe replacements of BPA.

TP021 Triclosan exposure delays development, alters sphingomyelin, and perturbs expression of genes

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Triclosan, a widely used bactericidal chemical used in consumer products, perturbs lipid metabolism and represses development in Daphnia magna under resource limited conditions. Triclosan is a known inhibitor of HR96 inhibitor, an ortholog of the xenobiotic-sensing nuclear receptors CAR/PXR found in mammals that control the regulation of phase I-III detoxification. HR96 has also been shown to regulate sphingolipid metabolism. Previous research in our group demonstrated the capacity of triclosan to increase sphingomyelin concentrations in D. magna associated with repressed development. Repression of sphingomyelin metabolism could be responsible for delaying development in neonates under low energy diets; thus increasing survival at the expense of reproductive development. RNA-sequencing was performed to determine changes in gene expression caused by 48-h exposure to 0.25 µM triclosan. Up-regulated genes include glutathione-s-transferases (GSTs), cytochrome P450s (CYPs), beta-mannosidase and mannose binding proteins. Examples of down regulated genes include cuticular proteins, carbohydrate sulfotransferases, glucose dehydrogenase, bile salt activated lipases, and ABC transporters. QPCR confirmation is currently underway as is further bioinformatic analysis of the pathways altered. In addition, the physiological and RNA-seq data generated indicates triclosan induces detoxification pathways, perturbs molting and growth, and alters energy metabolism, specifically lipid/sphingomyelin metabolism. Future research will work to associate specific pathways to perturbed development from a neonate to an adult. Overall, our research provides potential mechanisms as to why triclosan's toxicity is much worse than anticipated when exposure occurs in less than ideal environmental conditions.

TP022 Field and laboratory approaches to understanding testicular oocytes and gonadal development in smallmouth bass in relation to exposure to EACs

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In previous work we reported smallmouth bass populations in Northeastern Minnesota rivers and lakes with testicular oocyte (TOs) prevalence ranging from 7 to 59%, which is consistent with reports from other U.S. river systems. While it is often presumed that TOs are associated with exposure to endocrine active compounds (EACs), our analysis suggested only a weak relationship. To better understand gonadal development in smallmouth bass and how it might be influenced by exogenous estrogens, we conducted two laboratory exposures using 17-α-ethinyl estradiol (EE2). In experiment 1, smallmouth bass fry were exposed to 2 or 10 ng/L EE2 for 90 d beginning at swim-up, then reared for an additional 90 days in clean water. Treatment and control groups were subsampled intermittently for histological evaluation of gonadal development. In fish exposed to 2 ng/L EE2, 100% developed ovaries by day 63. The ovaries continued to develop even after cessation of EE2 exposure. In experiment 2, smallmouth bass fry were exposed to 0.11, 0.33, 1.0, or 3.0 ng/L EE2 for 100 days, and evaluated for gonadal development at days 90 and 120. In the group exposed to 3 ng/L EE2, 100% of fish developed ovaries, while all groups exposed to lower concentrations showed a roughly 50:50 female-to-presumed male sex ratio. No TOs were observed in any fish exposed to concentrations of EE2 lower than that which caused presumptive sex reversal. This result suggests that wild smallmouth bass TOs may not be caused by juvenile exposure to estrogenic compounds.

TP023 Steroidogenic genes in male mummichog exposed to model androgen 5α-dihydrotestosterone or 17α-methyltestosterone; an in vitro investigation

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Elucidating key events in adverse outcome pathways (AOP) is integral to strengthening biological links across multiple levels of organization. Current research has identified decreased plasma sex steroid hormones in mummichog, an estuarine killifish, after exposure to androgenic compounds, in conjunction with decreased egg size and reduction of plasma vitellogenin. However, the molecular cause is currently unknown. Potential impacts on the steroidogenic pathway in mummichog were assessed by exposing testis tissue in vitro to control (solvent only), 10^{-3}, 10^{-6} and 10^{-9} M of the non-aromatizable androgen 5α-dihydrotestosterone (DHT) and the aromatizable androgen 17α-methyltestosterone (MT) for 6, 12, 18 and 24 hours. A 24-hour preliminary in vitro (without androgen) was conducted to ensure RNA and gene expression integrity were maintained. There was no significant difference in RNA integrity across the 24-hour in vitro at 18^{O}C. RNA integrity from snap-frozen and in vitro tissue was also compared and no differences detected. A suite of genes including steroidogenic acute regulatory protein, 11β-hydroxysteroid

dehydrogenase and cytochrome P450 17A1 were analyzed for altered expression over 24 hours in the preliminary in vitro, with no difference in expression detected. Genes were normalized to β-actin. Comparison of aromatizable and non-aromatizable model androgen responses will increase knowledge of potential differences in androgen impact due to androgen classification and support AOP development.

TP024 Mixture effects of the analgesics dipyrone and acetaminophen in the catfish Rhamdia quelen

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Pharmaceuticals such as acetaminophen and dipyrone are prescribed in human medicine and have the potential to contaminate water and sediments via inputs from sewage treatment plants. Their impacts on humans and ecosystems are emerging issues in environmental health. Some pharmaceuticals can act as endocrine disruptors by changing gonads morphology and /or hormone levels in fish, for example. This study aimed to assess whether mixture of acetaminophen and dipyrone cause endocrine disruption by the expression of brain aromatase, testosterone and estradiol levels and oxidative stress in gonads of commercial fish Rhamdia quelen. Males and females were exposed separately to environmental concentrations of acetaminophen (2.5 µg/L), dipyrone (5 µg/L) and mixture (2.5 µg/L of acetaminophen + 5 µg/L of dipyrone) in a semi-static bioassay. These concentrations were used in previous studies. After 21 days of exposure, the animals were anesthetized and the blood was taken to hormone analyses. The hypothalamus was collected for the aromatase expression. The biochemical biomarkers as superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione S-transferase (GST), reduced glutathione (GSH) and lipoperoxidation (LPO) were analyzed in the gonads. In testis, GST activity was reduced by the pharmaceuticals mixture. Dipyrone also caused reduction in SOD and GST activities. No changes were observed in the GPx, GSH and LPO. Differing from males there was an increase of the SOD activity in the ovaries of animals exposed to the single drugs and to the mixture. The mixture caused LPO in female gonads, but no changes were observed in the GST, GPx and GSH. Estradiol and testosterone levels in males and females were not different from control. Brain aromatase expression in females was reduced by acetaminophen, dipyrone and the mixture. In males, aromatase expression was unchanged.The results demonstrated that the mixture of these two pharmaceuticals caused oxidative stress in female gonads and reduction of brain aromatase expression, suggesting an endocrine disruptor effect in females. The results also showed different responses in antioxidant system of males and females.

TP025 Toxicity and Thyroid Disrupting Effects of Halogenated Mixtures on Zebrafish Embryos

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Numerous halogens act as endocrine disrupting chemicals (EDCs) that can alter thyroid function. Members of this group include perfluorinated chemicals, commonly used in Teflon and food packaging, and flame retardants, used in a wide array of products such as clothing and electronics. Exposure of organisms to these EDCs can negatively affect growth and development. Therefore, there is a need to develop next generation, less toxic chemicals. The objective of this study is to further test these potential "safer" alternatives, singly and in mixtures. We used zebrafish embryos as our model organism due to its high structural and functional homology to humans and its suitability for early development studies. Three well known hazardous endocrine disruptors, perfluorooctanoic acid (PFOA), tris (1,3-dichloro-2-propyl) phosphate (TDCPP) and tetrabromobisphenal A (TBBPA), and two next generation chemicals, 9,10-Dihydro-9-oxa-10-phosphaphenanthrene 10-oxide (DOPO) and perfluorobutyric acid (PFBA), were tested both singly and in mixtures. First, we identified their LC50s under 96 hour exposures using zebrafish embryos. Next, we estimated their LC50 values in tertiary mixtures to identify any potential mixture effects. Our findings suggest that all mixtures tested display a partial addition effect; however, the next generation chemicals were less toxic in mixtures than their toxic counterpart. Finally, we are using an established pathway between thyroid disruption and swim bladder dysfunction to identify any thyroid disrupting effects caused by our chemicals of concern. We quantified changes in the surface area of the swim bladder as well as changes in expression levels of genes involved in thyroid dysregulation as a result of single and mixture exposures. Our preliminary findings from exposures to a mixture of PFOA, TDCPP and TBBPA showed reductions in swim bladder surface area, suggesting possible detrimental effects on the thyroid function during early development.

TP026 Molecular Signaling Pathways Elicited by 17-α-Ethinylestradiol in Intersex and Sex-reversed Japanese Medaka

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Water bodies receive effluents from waste water treatment plants, holding numerous endocrine disrupting chemicals. Fish exposure to these chemicals, particularly during sexual differentiation, can cause abnormal changes on gonadal development. 17-α-ethinylestradiol (EE2) is a well-studied synthetic estrogen agonist used in oral contraceptive formulations and commonly found in sewage effluents. Fish exposure to EE2 is known to elicit concentration dependent changes in gonads, ranging from the development of gonadal intersex to complete sex reversal. These gonadal changes have been well-studied in Japanese medaka, a laboratory fish model with genetic sex determination. The objective of this study is to understand the molecular signaling pathways involved in the development of gonadal intersex and sex reversal as a result of EE2 exposure. We exposed 9 dpf (days post fertilization) medaka larvae to 0, 20 and 200 ng/L EE2 for 30 days. These concentrations and length of exposure were chosen to elicit intersex and complete sex reversal, respectively. After 12 days of exposure and by the end of the sex differentiation period, male larvae were collected for next-generation sequencing (RNA-Seq) using Illumina HiSeq2500. The remaining males were exposed until 39 dpf then reared under controlled conditions until 90 dpf for histological analysis to validate the effects of these exposure regimes on gonadal development. RNA-Seq data analysis was performed using Tophat and Cufflinks to identify differentially expressed genes between different groups. Our findings suggest that exposure to EE2 induce direct (steroid hormone receptor-mediated) and compensatory changes to the hypothalamus–pituitary–gonadal–liver axis in a concentration-dependent manner. Results on the differentially expressed genes in intersex and completely sex-reversed males will increase our understanding on the molecular changes associated with the development of these gonadal abnormalities after EE2 exposure.

TP027 Effects of EACs in the Environment: Effectiveness of Freshwater and Saltwater Pond Remediation on Reducing Impact in Bermuda

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Indications of endocrine disruption and reproductive impairment in cane toads, killifish, and, diamondback terrapins in freshwater and marine ponds in Bermuda have prompted efforts to remediate polyaromatic hydrocarbons (PAH) and oxygenated PAHs (OPAH) within the pond sediments. A modified Tier 1 EDSP 21-day Fish Short-Term Reproduction Assay (FSTRA) was used to evaluate the effects of sediment exposure from freshwater and saltwater ponds in Bermuda on reproductive fecundity and endocrine function in fathead minnow (Pimephales promelas) and killifish (Fundulus heteroclitus), as well as, the effectiveness of remediation strategies. Baseline modified FSTRA assessment showed decreased reproductive fecundity, lower male body weight, and altered endocrinological measures of reproductive status were observed in both species. Higher plasma T levels in female minnows and 11-KT levels in both male and female minnows and female killifish exposed to freshwater and marine sediments, respectively. Decreased female E2 and VTG levels and gonadal CYP 19 (aromatase) activity were also found in sediment exposed females from both species. No effect on female 3/17-HSD activity was found in either species. Solar powered, diffuse, small bubble aeration was installed within one freshwater, and subsequently, in one saltwater pond. PAH, OPAH, and anaerobic degradation product levels were monitored over the course of 1.5 years and post-remediation modified FSTRA was used to assess remediation status of the freshwater pond. Reproductive performance and endocrine endpoints were similar to control sediment levels indicative of successful remediation. PAH, OPAH, and anaerobic degradation product levels are being monitored in the saltwater pond. Modified FSTRA will be used similarly to assess remediation success.

TP028 Cadmium and BPA in vivo exposures alter endocrine related genes in the freshwater snail Physa acuta. New biomarker genes in a new model organism

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The freshwater snail Physa acuta is a sensitive organism to xenobiotics and appropriate for aquatic toxicity testing in environmental studies. There is scarce genomic information on P. acuta so in this work we identify several genes related with the hormonal system to evaluate the effects of Cadmium (Cd) and Bisphenol A (BPA), pollutants frequently found in aquatic environments. Endocrine-disrupting chemicals (EDCs) can affect the synthesis or secretion of hormones and alter their endogenous functions. We have selected two compounds with known endocrine-disrupting activity in vertebrates to analyze their potential effect on the Gastropoda endocrine system. The transcriptional activity of the endocrine related genes estrogen receptor (ER), estrogen-related receptor (ERR), and retinoid X receptor (RXR) were analyzed in P. acuta exposed to Cd and BPA. Real time reverse transcriptase-polymerase chain reaction (qRT-PCR) analysis showed that Cd and BPA presence altered the expression pattern of ER, ERR, and RXR mRNAs, suggesting a putative mode of action that could explain the endocrine disruptor activity of these xenobiotic at a molecular level on Gastropoda. These data provide, for the first time, information about the effects of Cd and BPA on the endocrine system of Gastropoda and suggest that ER, ERR and RxR genes could be a potential biomarkers of exposure to compounds with activity as endocrine disruptors. Acknowledgements: Funded by CTM-2015-64913-R from the Ciencias y Tecnologías Medioambientales program (SPAIN).

TP029 Advantages and Challenges for Determining Mode of Action of Industrial Chemicals: A Case Study with Alkylphenol and Alkylphenol Ethoxylates

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Determining the mode of action for environmental contaminants is increasingly important in the field of environmental toxicology for several reasons including, but not limited to: 1) potential future use of predictive toxicity methodologies which are based on molecular initiating events and modes of toxic action, 2) refining the assessment of the hazards of environmental mixtures in the environment, and 3) consideration of proposals for hazard based approaches to regulating chemicals based on perceived mode of action. While the mode of action of pharmaceuticals and pesticide active chemistries may be relatively straight-forward based on their design for efficacy with a selection toward minimizing non-target effects, industrial chemicals present unique challenges. This case study will examine Alkylphenols (APs) and low mole alkylphenol ethoxylates (APEs), which are environmental degradants of the higher mole APEs used as detergents, emulsifiers, wetting agents and dispersing agents in industrial chemical formulations. AP and low mole APE are known to have weak estrogenic activity; however, this is not the only or even the primary mode of action that is linked to adverse effects in environmental species Several lines of evidence will be presented that can be used to elucidate the mode of action of these and other industrial chemicals. High through-put toxicity screening, acute to chronic ratios, weight of evidence evaluations based on chronic toxicity data from various taxa (i.e. mammalian, fish, invertebrate and plant), and "omic" data will be used to make the case that for many industrial chemicals, a "lead" toxic mode of action may be hard to identify. Recommendations for determining modes of action for industrial chemicals and implications for regulatory aspects will be discussed.

TP030 Screening, Prioritizing, and Classifying EDCs: A comparison of international efforts to identify endocrine disrupting chemicals

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To date, a wide variety of organizations across multiple sectors around the world have published reports and communications proposing lists of chemicals that they have labeled as endocrine disrupting chemicals (EDCs) or suggested as potential EDCs. For example, government agencies from multiple countries have established their own priority chemical lists, screening programs, and/or knowledge bases to assist with ongoing research and pending management measures. Some industry bodies have also reviewed the topic and published their own lists for self-regulation within the respective sector. The intended purpose, selection criteria used to create these lists, and details included differ considerably among them. In this study, we have reviewed and consolidated in total over fifteen existing lists in the public domain. In particular, we aim to step back and gain an overview of the global efforts being made to identify and classify EDCs and to establish which chemicals have received the most consensus and evidence for being EDCs or should be prioritized for assessment in the foreseeable future. From the evaluation, we noted that while many lists have already been heavily developed and publicized, others are planned or currently in early development stages. Clarity of the selection criteria was also found to be varied, with some lists discussing the exact methodology used and others citing some form of expert consultation or not disclosing clear criteria. Multi-stakeholder consultation (through government, business, and/or community input) also occurred during the development of some, especially those that have direct regulatory impacts. Other differentiating factors include whether a list is static or changeable, homogenous or further sub-classified, and EDC-focused or more universal. Our comparison demonstrates that significant resources have been and are being invested into identifying and understanding EDCs, as reflected by the number and diversity of these lists. Simultaneously, it also highlights inconsistencies in methods being used, the lack of input from developing and transition countries, and the need for further clarification of the meaning and purposes of the lists.

Birds as Indicators of Environmental Change: Molecular to Population Effects of Contaminant Exposure and other Stressors

TP031 Levels of legacy and emerging organohalogenated compounds in Northern goshawk and White-tailed eagle nestlings from different latitudes in Europe

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Since the ban of several organohalogenated compounds (OHCs), industries have produced unrestricted alternatives that are also emerging in the environment, including birds. To assess to which degree these compounds are present in birds of prey, nestlings of Northern goshawk (NG; Accipiter gentilis) and White-tailed eagle (WTE; Haliaeetus albicilla) were investigated for exposure to legacy and emerging OHCs. Birds were sampled over a latitudinal gradient in Spain (NG: Murcia at 38°N) and in different locations in Norway (NG: Trøndelag at 63°N, Troms at 70°N; WTE: Smøla at 63°N, Steigen at 67°N). Blood and body feathers were taken as non-destructive sampling methods. Polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), dichlorodiphenyltrichloroethane and metabolites (DDXs), chlordanes (CHLs), hexachlorobenzene (HCB) and hexachlorohexanes (HCHs), together with emerging compounds, such as novel brominated flame retardants (NBFRs), dechlorane plus (DP) and phosphorus flame retardants (PFRs), were analysed using gas chromatography-mass spectrometry. The total OHC load in feathers of NG was twice as high in the northernmost location Troms (280 [181-518] ng g^{-1} dw) as in Trøndelag (93 [52-165] ng g^{-1} dw) and Murcia (103 [55-198] ng g^{-1} dw). This pattern differed in plasma, as Trøndelag had the highest OHC load (11 [1.8-40] ng mL^{-1}), followed by Troms (7.6 [1.4-35] ng mL^{-1}) and Murcia (3.2 [1.2-21] ng mL^{-1}). In WTE, birds from Steigen had the highest OHC load in both matrices, with plasma concentrations twice as high as in Smøla (11 [6.3-115] and 4.1 [1.8-15] ng mL^{-1}, resp.). In both species, PFRs were by far the major contributors to the total OHC load in feathers (36-84%). Other major contaminant groups were DDXs and PCBs (or HCHs in Murcia). The most dominant compounds of each group found in feathers of both species were tris(1-chloro-2-isopropyl)phosphate, p,p'-DDE, CB153, BDE47, oxychlordane (OxC) and β- and γ-HCH. In plasma, DDX and PCBs were the main contaminant groups (≥95% of the total OHC load) and CB153, CB180, p,p'-DDE, OxC, BDE99, BDE47 and β-HCH were the dominating compounds. PFRs in plasma were only detected in WTE from Steigen. In general, feather concentrations exceeded those in plasma, especially PFRs. This could be due to accumulation in feathers during feather growth or external contamination. Also, fewer compounds were detected in Spain than in Norway, suggesting further investigation of geographical exposure patterns.

TP032 Assessing Cholinesterase (ChE) activity in the Humboldt penguin (Spheniscus humboldti) from Punta San Juan de Marcona, Ica, Peru

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The use of current used pesticides with associated misuse and overuse in operational applications have resulted in the contamination of land, freshwater and coastal areas of the Pacific coast of South America. The toxic effects and persistence of these compounds in the marine environment are scarcely known in Peru. Exposure to organophosphorus (OP) and carbamates (CB) can inhibit the function of acetylcholinesterase (AChE), the enzyme responsible for neurotransmission, causing mortality or lethal effects in organisms. With the aim to determine baseline information on the normal activity of cholinesterase (ChE) in free-ranging Humboldt penguins (Spheniscus humboldti), we assessed ChE activity levels and whether body condition and sex of individuals are factors influencing ChE in penguins. Blood samples were collected from 17 males and 18 adult females in 4 areas of the Reserve Punta San Juan in Ica, Peru. Sampled penguins were considered clinically healthy. Samples were centrifuged and serum collected for biochemical analysis. We used generalized linear mixed models to explore the influence of sex, body condition and sampling location on the values of ChE. Body weight was included as a covariate, and the area as a random factor. The enzymatic activity was expressed in units (U) per L of serum (i.e. 1 U is a µmol of substrate hydrolyzed per minute). Mean concentrations of ChE ranged from 1248 ± 264 U/L (1.25 ± 0.3 U/mL) to 1413 ± 196 U/L (1.40 ± 0.2 U/mL), with a minimum concentration of 618 U/L (0.60 U/mL). Males had lower cholinesterase levels relative to those detected in females. However, the relationship between body weight and ChE should be interpreted with caution. While the association of ChE activity found in our penguins to ChE inhibiting pesticides or anti-cholinesterase compounds is scarcely understood due to the lack of background values and thresholds related to pesticides applications or chemical assaults in the region, our study reveals the first baseline that can be used as reference for ChE activity as a potential biomarker in Humboldt penguins as well as for wildlife and ecotoxicological risk-assessment. The range of ChE activity found here may be considered as basal levels for non-exposed penguins or penguins relatively exposed to low levels of pesticides (OP or CB) from unidentified local sources, highlighting the use of this seabird species as a sentinel to monitor the state of the health of the coastal-marine ecosystem along the Peruvian coast.

TP033 Useful biomarkers in sentinel European starlings (Sturnus vulgaris) exposed to vehicle exhaust emissions

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The health effects of air pollutants have been established in common laboratory species for many individual compounds, and at excessive (unnatural) concentrations. Epidemiological studies are identifying relationships between inflammatory disease syndromes in humans and air pollutants such as oxides of nitrogen and sulphur, and fine (respirable) particulate matter. There is very little information available on the effects of realistic pollutant mixtures on wildlife, particularly from experimental exposures. Wild-caught, adult European starlings (Sturnus vulgaris) were divided into two groups of approximately 30 birds each to study the effects of vehicle emissions on wild passerines. Experimental and reference groups were housed and managed under the same conditions, controlling for the effects of vehicle noise, lighting and temperatures. The experimental group was exposed to vehicle exhaust diluted to simulate maximum ambient urban air concentrations, vented through their enclosure for five hours per day for one month. Body condition (using scaled mass index), cell- and antibody-mediated immune responses, and hormonal responses were assessed as non-lethal indicators of toxicologic effects, whereas hepatic detoxification enzymes (EROD), measures of oxidative stress (TBARS, GSH) and the histopathology of selected tissues provide insight into other possible biomarkers. Early results indicate a significantly reduced response to the PHA skin test of cell mediated immunity. Other responses in the contaminant exposed birds compared to the controls will be presented based on these hormonal and toxicological biomarkers. As well, we describe the experimental setup, and provide recommendations for future studies of exposure to air pollutants.

TP034 The Use of Homing Pigeons as a Biomonitor for Atmospheric POPs in Guangzhou, China

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With the worsening air quality in China, it is essential to monitor the contaminants of concern in the atmosphere, especially in the megacities. Traditional atmospheric monitoring, such as using passive air samplers provides the concentration data in gaseous phase and particulate phase, but lacks the consideration about bioavailability, not to mention assessing toxicity risk of long-time exposure. Instead, biomonitoring with homing pigeons raised in urban area could be served as a suitable biomonitor of air pollution in cities. First, the homing ability makes homing pigeons live in their birth places during lifetime and keep exposing in air in the target area. Second, every homing pigeon has a foot ring recording its birth information, and usually they can live more than ten years. Last, more than 80,000 homing pigeon lofts are registered in China, spreading almost all cities in 34 provinces. In this research, 29 homing pigeons in Guangzhou urban area were collected and grouped to 1-, 5-, 10-year age groups. After necropsy, histopathological assessment was conducted for lung and liver. Concentrations of a variety of persistent organic pollutants (POPs), including polycyclic aromatic hydrocarbons (PAHs), organochlorine pesticides (OCPs), polychlorinated biphenyl (PCBs) and halogenated flame retardants (HFRs) in lung, liver and fat tissues were measured. In addition, drinking water and three types of pigeon food were sampled. There was a positive correlation between contaminant concentrations and the age of pigeons. Except of few PAHs, no other target POPs were detected in pigeon food and water, indicating these POPs were likely accumulated in the organisms through respiration. Within the same age group, concentrations of HFRs in lung tissue were significantly higher than those in liver tissue, verifying the respiration was the dominant route of exposure rather than diet. For the homing pigeons with high PAH concentrations in lung tissue, pathological responses were also observed. Female homing pigeons in the 5 and 10 year groups have significantly lower POPs concentrations in fat tissue than the males, probably because the POPs were reduced from the females by laying eggs. Compared with atmospheric monitoring data, biomonitioring data using homing pigeons provides a reference for assessing the respiration exposure risk of POPs to the residents in study area.

TP035 Effects of PCB 126, PCB 77 and Two Environmentally Relevant PCB Mixtures on the Gonadotropin Releasing Hormone System in Hatchling Japanese Quail

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Gonadotropin releasing hormone-I (GnRH-I) is a key component of reproductive function of vertebrates, with birds being no exception. Stimulation of GnRH-I producing neurons causes release of GnRH-I into the hypophyseal portal system followed by stimulation of the release of follicle stimulating hormone (FSH) or luteinizing hormone (LH) from the anterior pituitary. During non-breeding periods the hypothalamic-pitiuitary-gonadal axis (HPG) is relatively quiescent, but is stimulated by seasonal changes in daylength leading to activation of reproductive function. Adequate function of the HPG axis is at hatch is particularly important in precocial species such as the Japanese quail (Coturnix coturnix japonica) as it ensures appropriate sexual development and growth for reproduction. The purpose of this study was to determine if embryonic exposure to PCB 126, PCB 77 and two environmentally relevant PCB mixtures affected the development of the HPG axis and if there were detectable effects in hatchlings that could potentially relate to impaired reproductive function in adults. Fertile eggs from Japanese quail (n=30/group) were injected with the PCBs prior to incubation. The concentrations injected resulted in dose response curves in all treatments, except for PCB 77 injected eggs. Birds were sampled at hatch, brains were dissected rapidly and frozen over dry ice to retain the integrity of the brain shape for accurate hypothalamic punches to be performed. Hypothalamic GnRH-I was measured using an EIA specific for GnRH-I, the active form in avian species. As in previous studies, hypothalamic GnRH-I was sexually dimorphic. Response to the PCBs was non-linear, and followed an inverted U response, suggesting that embryonic survival at higher doses was also a factor in the response and the more sensitive embryos did not survive. These results point to potential actions of PCBs on hypothalamic systems that may be independent of thyroid system impacts of the PCBs. This work was supported by the U.S. Fish and Wildlife and the Hudson River Natural Resource Trustees. The conclusions and opinions presented here are those of the authors and do not represent the official position of any of the funding agencies, the Hudson River Trustees, or the United States of America.

TP036 Effects of PCB77 on development of the Bursa of Fabricius at hatch in the tree swallow (Tachycineta bicolor)

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The Bursa of Fabricius of hatchling birds is an important component of the immune system for production and maturation of B-cells. Sex hormones such as estrogens and androgens play an important role in bursa development, as can endocrine disrupting compounds. The purpose of this study was to determine if embryonic exposure to PCB 77, a dioxin-like PCB, would cause anatomical changes in the bursa of hatchling male or female tree swallows. Fertile tree swallow eggs at approximately embryonic day 2.5 were injected with 2.0uL of PCB 77 and placed back in the nest to develop. Eggs remained in place until two days prior to hatch, then were transferred to the laboratory. Control eggs were treated identically. All eggs were checked for development twice before removal to the laboratory. Samples were collected within four hours of hatch and fixed in neutral buffered formalin, then stained with hematoxylin. There were changes in bursa, follicle and lumen size associated with treatment. This work was supported by the U.S. Fish and Wildlife and the Hudson River Natural Resource Trustees. The conclusions and opinions presented here are those of the authors and do not represent the official position of any of the funding agencies, the Hudson River Trustees, or the United States of America.

TP037 Tree swallow chromosomal damage across the Great Lakes: An application of a multi-level model to ecotoxicology

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Tree swallows (Tachycineta bicolor) may serve as a bioindicator because toxicants accumulate in the species. These toxicants also hold the potential to adversely affect the tree swallow and may potentially damage DNA. Chromosomal or DNA damage can be measured by examining genome size coefficients of variation (DNA CV) using flow cytometric techniques. We sought to examine the amount of chromosomal damage within tree swallows across the Great Lakes and we collected nestlings from 69 sites in the Great Lakes basin. These sites included multiple sites within 27 Areas of Concern (AOCs) and 9 nearby non-AOC sites. Our data contained multiple levels of information and accounting for this information required considering the hierarchical nature of the data. Specifically, we developed a multi-level (or hierarchical) model to account for the variability and covariates within replicates, within nestling, within nest box, within site, and within AOC/meta-site. This model also allowed us to account for pseudo-replication. Using the multi-level model, we were able to estimate the DNA CV values at different levels of inference. We specifically found that some sites had greater chromosomal damage than our reference sites. We also found important covariates at different levels within the model. In addition to our specific results, we also discuss how multi-level models may be used to address other situations found in ecotoxicology.

TP038 Concentrations and spatial patterns of organic contaminants in tree swallows at US and Binational Great Lakes Areas of Concern (AOCs), 2010-2015

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Tree swallows (Tachycineta bicolor) were sampled across the Great Lakes basin in 2010-2015 to provide a system-wide assessment of current exposure to organic contaminants as part of the Great Lakes Restoration Initiative (GLRI). These results provide information identified as critical by regulators to assess the ‘Bird or Animal Deformity or Reproductive Problems’ Beneficial Use Impairment (BUI). Eggs were collected from 69 sites across all five Great Lakes, including 27 Areas of Concern (AOCs), some with multiple sites, and ten sites not listed as an AOC. Concentrations of organic contaminants in eggs were quantified and compared to background and reproductive effect thresholds. At approximately 30% of AOCs, tree swallow eggs had geometric mean total polychlorinated biphenyls (PCBs) concentrations at or below average background concentrations (0.34 mg/g wet wt.). Mean concentrations at 78% of AOCs were below the mean at the most contaminated non-AOC location (1.30 mg/g wet wt.). The AOC with the highest level of PCBs in eggs was Waukegan Harbor, IL (geometric mean = 7.30 mg/g wet wt.) followed by Lower Green Bay and Fox River, WI (2.6 mg/g wet wt.) and Kalamazoo (2.2 mg/g wet wt.). Exposure to polybrominated diphenyl ethers (PBDEs) was minimal, and only 3 of 27 AOCs and one non-AOC had geometric mean concentrations that exceeded background for tree swallows (96 ng/g wet wt.). Concentrations of both PCBs and PBDEs were 10 to >20 times below the lower limit for hatching effects. In contrast, geometric mean concentrations of dioxin and furan (PCDD-F) toxic equivalents (TEQs) at Saginaw River and Bay AOC (242 pg/g wet wt.) and Midland, MI (475 pg/g wet wt.), a non-AOC site upstream, exceeded the lower limit for hatching effects (181 pg/g wet wt. PCDD-F TEQs). All other sites had geometric mean concentrations of PCDD-F TEQs below background levels (87 pg/g wet wt. PCDD-F TEQs). Other organic contaminants, including p,p'-dichlorodiphenyldichloroethylene (DDE), mirex, heptachlor, and chlordane, were at or below background concentrations, or did not differ between AOCs and non-AOCS. With some exceptions, both at the site or individual sample level, there seems to be only minimal exposure (at or below background) to the organic contaminants measured in the Great Lakes Basin.

TP039 Transcriptomics, contaminants, and other biomarkers in tree swallow (Tachycineta bicolor) nestlings from the Great Lakes

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Polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), dioxins, and a variety of pesticides and other contaminants of emerging concern are significant issues in the Great Lakes Areas of Concern (AOC). Tree swallow (Tachycineta bicolor) nestlings were collected from 27 AOCs and nine nearby non-AOC sites, with multiple sites on each of the five Great Lakes, from 2010 to 2015. Contaminant analyses and biomarkers were examined in all nestlings collected. In birds, hepatic cytochrome P450 1A4 (cyp1a4) and cytochrome P450 1A5 (cyp1a5) gene expression have been shown to be good indicators of an induced aryl hydrocarbon receptor (AHR) pathway, whose ligands include dioxins, PAHs, and PCBs. Additionally, their expression is generally proportional to the commonly utilized surrogate for AHR induction, ethoxyresorufin-O-deethylase (EROD) activity. Among our samples, we observed population-specific differences in the strength of the correlation between AHR pathway induction and PCB and PAH concentrations, particularly at Waukegan Harbor, IL. Unlike other PCB-contaminated AOCs, we found that EROD activity was only moderately induced at Waukegan Harbor, despite mean nestling PCB concentrations of 6 ppm and high total PAHs in stomach contents. Additionally, transcriptomic analysis was performed for a subset of samples, including nestlings from Manistique River, MI, River Raisin, MI, Saginaw River, MI, Waukegan Harbor, and four other non-AOC sites. RNA-Seq library construction and sequencing used Illumina chemistries. The de novo transcriptome was assembled using the Trinity platform, followed by RSEM for expression estimation and EdgeR for differential expression analysis. Transcriptomic analysis indicated that cyp1a4 and cyp1a5 expression at Waukegan Harbor was only about one third of that observed at River Raisin, although the PCB concentrations from Waukegan Harbor were three fold higher than those from River Raisin. Other population differences in gene expression, and their correlations with other biomarkers and environmental chemistry will also be discussed.

TP040 Transcriptome profiling of American kestrels exposed in ovo to two persistent organic pollutants -SCCPs and TBBPA-BDBPE

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The increased use of alternative flame retardants and related products and their reported persistence in the environment have resulted in greater bioaccumulation in wildlife located near point sources and in more remote locations. However, few data are available on the potential adverse effects of these compounds in the exposed animals. Here we report on an investigation into genomic effects of two such persistent organic pollutants (POPs) – short-chain chlorinated paraffins (SCCPs) andtetrabromobisphenol A bis[2,3-dibromopropyl ether] (TBBPA-BDBPE; CAS 21850-44-2) – on a predatory bird species, the American kestrel(Falco sparverius). SCCPs are emerging POPs identified by the Stockholm Convention and by the U.S. Environmental Protection Agency as chemicals of concern due to potential long-term risks from exposure. These highly resistant additive chemicals are used in metal lubricants and coolants in metal cutting, and as plasticizers and flame retardants in plastics and paints. Similarly, TBBPA-BDBPE, a derivative of TBBPA,is an additive flame retardant that is used in a wide range of plastic products, resins, textiles, paints, and household electronics. Although TBBPA-BDBPE was introduced as a possible replacement for decabromodiphenyl ether (decaBDE), it may be as persistent and has similar environmental transport mechanisms. In this study, RNA-Seq analysis was performed to identify differentially expressed genes in American kestrels exposed in ovo to environmentally relevant concentrations of SCCPs and TBBPA-BDBPE. Our goal was not only to identify dysregulated genes, but also to gain a better understanding of kestrel genomics. A transcriptome was constructed de-novo and benchmarked, obtaining 25,000 annotated isoforms pertaining to nearly 17,000 unique genes. Differential expression analysis of SCCP-exposed males and females together, identified significant changes in 673 genes (adjusted p-value < 0.05), with 3 genes exceeding log2 fold changes. In TBBPA-treated hatchlings, 4 genes exceeded a log2 fold change over vehicle control birds. Analyses of differential expression by sex for each compound, increased the number of genes exceeding log2 fold change, suggesting significant differences in the responses of male and female American kestrels to both of these POPs.

TP041 Identifying adverse effects on neuroanatomy of hatchling American kestrels exposed to two novel brominated flame retardants

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Each year, new chemicals are introduced to the market. The Canadian federal government's Chemicals Management Plan (CMP) has identified priority chemicals, including multiple flame retardants, for risk assessment and management. Techniques for assessing these chemicals have generally not considered their potential impacts on the brain. Some flame retardants accumulate in the brain, and the brain is known to be sensitive to physiological perturbations including contamination. We used an established technique, neuroanatomy, in a novel context, ecotoxicology. We exposed American kestrels (Falco sparverius), key top predators of terrestrial ecosystems, in ovo to one of two commonly used brominated flame retardants (BFR): 2-ethylhexyl-2,3,4,5-tetrabromobenzoate (EHTBB also abbreviated as TBB; CAS 183658-27-7), or bis(2-ethylhexyl)-2,3,4,5-tetrabromophthalate (TBPH; CAS 26040-51-8). Controls received vehicle (safflower oil) only. We sectioned 40-µm slices throughout the brain of hatchling kestrels using a cryostat, followed by Nissl staining to label all the cells and delineate targeted brain regions. We measured the volumes of the hippocampus and the telencephalon in the left and right hemispheres. The hippocampus, a region in the telencephalon, plays a crucial role in spatial memory and navigation in birds, and dysfunction in the hippocampus could affect their survival and reproduction. BFR are especially likely to impact the hippocampus because of their known effects on thyroid hormones that influence hippocampus development and neurogenesis, and on sex steroids for which the avian hippocampus expresses androgen and estrogen receptors. In addition to determining whether hippocampus volume relative to the telencephalon is affected by BFR exposure, we determined whether whole telencephalon volume and symmetry between the hemispheres were affected. This project links chemical exposure with potential neurological effects in birds. These changes could have an impact on behaviour at the individual level, and ultimately, affect populations.

TP042 Mercury concentrations in Double-crested Cormorant chicks across Canada

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Spatial patterns of mercury (Hg) have been found in freshwater ecosystems across Canada for many taxa including fish and birds. Hg biomagnifies in aquatic food chains and can reach high concentrations in fish-eating birds. However, it is often hard to sample a representative population size of adult birds to monitor concentrations of contaminants over a large spatial scale. Moreover, adult birds can migrate and can show a contaminant profile that may not be entirely representative of local resources of the monitored ecosystems. The aim of this study were (1) to develop a model to estimate Hg concentrations in breeding adults by using chicks as proxy and (2) to determine if there was a spatial pattern of Hg in piscivorous birds. Double-crested Cormorant (Phalacrocorax auritus) chick feathers were sampled at 19 sites across Canada (n = 5 chicks / site). Adult tissues (freshly grown feathers and blood) were sampled at five of those locations to establish correlations between tissues and age classes. We found a good correlation of Hg concentrations between adults and chicks and between adult tissues. We developed a model to estimate Hg concentrations in adults across Canada using chicks as proxy. We found an increase in Hg concentrations with latitude, but not with longitude. Our study shows that chicks can be a suitable proxy for monitoring mercury concentrations in adult fish-eating birds. Future work will include an analysis of birds across North America.

TP043 Determining Baseline Mercury Concentrations in Seabird Eggs in Lake Melville, Labrador, Canada Before Flooding of the Muskrat Falls Hydroelectric Dam

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Nalcor Energy is currently constructing the Muskrat Falls hydroelectric dam on the Churchill River in Labrador, Canada. Flooding of the reservoir is expected in 2017-18. At the environmental assessment hearings which preceded the project, concerns were raised by the Nunatsiavut Government and the Government of Canada about downstream increases in aquatic methylmercury in Lake Melville, as a result of dam construction. Nalcor Energy discounted these concerns. This project began in 2013, to determine the baseline mercury concentrations in the eggs of marine birds breeding in Lake Melville, prior to flooding of the Muskrat Falls reservoir. We collected eggs of ring-billed gulls (Larus delawarensis) and common terns (Sterna hirundo) on islands in western Lake Melville in 2013-15. These eggs were analysed individually for total mercury, and in 2015 for stable carbon and nitrogen isotopes ($\delta^{13}C$ and $\delta^{15}N$, respectively). We found no differences in mean egg mercury concentrations among islands or among years for either ring-billed gulls or common terns. The geometric mean of egg mercury concentrations in common tern eggs (0.92 µg/g, dry wt.) was significantly greater than in ring-billed gull eggs (0.77 µg/g), while the variance in mercury concentrations was greater in the gull eggs. In eggs collected in 2015, $\delta^{15}N$ was analysed to investigate the trophic patterns of the gulls and terns. Comparing $\delta^{15}N$ data (mean ± SD) between ring-billed gull eggs (10.5 ± 1.3‰) and common tern eggs (12.5 ± 0.5‰) indicated that the gulls foraged over a wider trophic range, and on average at a lower trophic level than the terns. Mercury concentrations in the gull eggs were unrelated to either $\delta^{13}C$ or $\delta^{15}N$. However, in the common tern eggs, both $\delta^{13}C$ and $\delta^{15}N$ were negatively associated with mercury concentrations. Current mercury levels in the Lake Melville gull and tern eggs pose little risk of adverse effects on reproduction. This study provides baseline data prior to the flooding of the Muskrat Falls reservoir, which we will use to assess the amount and duration of subsequent mercury increases after flooding.

TP044 Methylmercury Effects on Bird Reproduction: Critical Review and Identification of Toxicity Reference Values

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Effects of Hg on birds have been studied extensively and with increasing frequency in recent years. We conducted a comprehensive review of MeHg effects on bird reproduction, evaluating laboratory and field studies in which observed effects could be attributed primarily to Hg. Our review focused on exposures via diet and maternal transfer in which observed effects (or lack thereof) were reported relative to Hg concentrations in diet, eggs, or adult blood. Applicable data were identified for 25 species. From this data set, we identified ranges of toxicity reference values (TRVs) suitable for risk assessment applications. Typical ranges of Hg effect thresholds are approximately 0.2 to 1.5 mg/kg in diet, 0.08 to >0.3 mg/kg-day on a dose basis, 0.6 to 2.6 mg/kg in eggs, and 1.7 to >6.7 mg/kg in parental blood (all concentrations on a wet weight basis). For Hg in avian blood, this review represents the first broad compilation of relevant toxicity data. For dietary exposures, the current data support TRVs that are higher than older, commonly used TRVs. The older diet-based TRVs incorporate conservative assumptions and uncertainty factors that are no longer justified, although they generally were appropriate when originally derived, due to past data limitations. The egg-based TRVs identified from this review are more similar to other previously derived TRVs but have been updated to incorporate new information from recent studies. While important research needs remain, a key recommendation is that species not yet tested for MeHg toxicity should be evaluated using toxicity data from tested species with similar body weights.

TP045 Elucidating Biomarkers of Developmental Toxicity Associated with Pharmaceutical Pollutants

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Pharmaceutical pollutants are emerging contaminants of concern as low concentrations of therapeutic drugs are detected in a variety of water sources around the world. Few published studies address the toxicity of these compounds from chronic exposures at concentrations well below therapeutic doses. Even less is known about how these pollutants effect susceptible populations, such as the developing embryo. Many pharmaceutical agents found in water sources are endocrine disruptors, which can disrupt the hypothalamus-pituitary-adrenal axis, or stress axis. Early life stress in the form of environmental contaminants can have profound effects on both development and regulation of body functions later in life. Therefore the focus of this research is on phenotypic anchors associated with the stress axis in an avian model. Fertilized chicken eggs were injected with 0.0001, 0.00001, or 0.000001 mg/mL in DMSO of fluoxetine, fenofibrate, or norethisterone, which represent different pharmaceutical classes that have been detected most abundantly in recent water sampling studies. Upon hatching, collected endpoints included open-field activity as a general marker of arousal and body and organ weights as markers of developmental toxicity. Pre-hatching mortality indicated thatfor eggs that did not hatch, most embryos stopped developing within the first week of incubation, a period characterized by rapid energy utilization for organogenesis. Open-field activity suggested that fluoxetine and norethisterone decreased the arousal of hatchlings at all doses, as compared to the control. No significant changes in organ weights were observed, although there was wide variability in the weights of spleens and bursas of hatchlings exposed to various concentrations of fenofribrate and norethisterone. These phenotypic changes indicate that exposure to pharmaceutical pollutants at environmental concentrations can impact the development and behavior of chickens. Future analysis will include plasma corticosterone levels and brain and liver glucocorticoid receptor concentrations to anchor developmental indicators with changes to the stress axis.

TP046 Competitive Binding of Persistent Organic Pollutants to the Thyroid Hormone Transport Protein Transthyretin in Glaucous Gull (Larus hyperboreus)

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The glaucous gull (Larus hyperboreus) is one of the largest avian top predators in the Arctic. High levels of persistent organic pollutants (POPs) and their metabolites have been detected in the glaucous gull. Several studies indicate that these high levels can contribute to detrimental effects, and one possible mechanism is that chemicals interfere with the endocrine system. Thyroid hormones (THs) are important for thermogenesis, reproduction, growth and differentiation, and are transported in the circulation system of glaucous gull mainly bound to the transport proteins globulin, albumin and transthyretin (TTR). The present study used molecular modeling to construct homology models of TTR in glaucous gull and docked several well-known and emerging POPs in the models to predict binding affinities of POPs to the TH binding site in glaucous gull TTR. The models predicted that a large group of structurally diverse compounds would bind to glaucous gull TTR, mostly halogenated compounds but also not halogenated compounds. Among the predicted TTR binders were polybrominated diphenyl ethers (PBDEs), polychlorinated biphenyls (PCBs) and their metabolites, different brominated, chlorinated and phosphorus flame retardants, perfluorinated compounds (PFCs) and other emerging compounds. The presence of functional groups in the compounds were predicted to increase the binding affinity, and if the functional groups were ionized the binding affinity was predicted to further increase. However, the presence of functional groups were not required for the compounds to bind to TTR. The models also predicted that brominated analogues had higher affinity to TTR than the corresponding chlorinated analogues. Several of the brominated compounds, PFCs and THs had comparable binding affinity to TTR. Many of the compounds have been found in the glaucous gull or in the Arctic environment and organisms. The results indicated that competitive binding of multiple POPs with THs for TTR in glaucous gull can potentially disrupt the circulation of THs and possibly affect the TH homeostasis and TH-dependent functions.

Existing and Emerging Contaminants in Changing Arctic Environments

TP047 Accumulation of PBDEs of in Stranded Harp (Pagophilus groenlandicus) and Hooded Seals (Cystophora cristata) from the Northeastern United States

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Polybrominated diphenyl ethers (PBDEs) are highly lipophilic components of brominated flame retardants that are environmentally persistent and bioaccumulate. PBDEs are effectively taken up from the gastrointestinal tract and accumulate mainly in fat depots (blubber) and liver tissues. Species inhabiting Arctic and sub-Arctic regions, such as seals, can have upwards of 30% of their body mass composed of blubber. When those blubber stores are mobilized for energy, they are simultaneously releasing stored toxicants into circulation causing them to be more bioavailable. Most studies looking at accumulation of PBDEs in seals have focused on harbor and grey seals with few looking at harp and hooded seals. In this study, we analyzed accumulation of PBDEs in seal blubber from 22 stranded harp and 9 stranded hooded seals sampled along the northeast coast of the U.S. (1999-2010) and determined the PBDE congener fingerprint for each individual. PBDEs were extracted from blubber sub-samples using gel permeation chromatography and analyzed using GC-MS. The five most environmentally relevant PBDEs were targeted: BDE-47, -99, -100, -153, and -154. Total PBDE concentrations were calculated for each individual using the relative response factors determined from a calibration curve generated using certified reference materials. The congener fingerprint (concentrations and % totals for each congener) was determined for each individual. Preliminary results show ?PBDEs in both species ranging from 65.15- 149.74 ng/g lw. BDE-47 is the most abundant congener found in all samples comprising upwards of 50% of the total PBDES present. The results of this study will aid in understanding the effects of PBDEs on harp and hooded seals being that both of these species are reliant on blubber stores during all life stages.

TP048 Temporal and spatial trends of polychlorinated naphthalenes (PCNs) in ringed seals from the Canadian Arctic

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The ringed seal is the most abundant Arctic pinniped with a circumpolar distribution and has been a key biomonitoring animal for examining spatial and temporal trends of persistent organic pollutants (POPs) in the Arctic since the 1970s. Polychlorinated naphthalenes are widespread environmental contaminants that have been reported in air samples and marine mammal tissues from the Arctic although spatial and temporal data are much more limited compared to most POPs. While intentional production and use of PCNs stopped in the 1970/80s they remain in old products such as capacitor fluids, engine oil additives, and electrical insulators. Current PCN emissions are mainly as by-products of combustion such as waste incineration and coke production from coal. The main objective of this research was to investigate the presence and the temporal trends of PCN in ringed seals from the across the Canadian Arctic. Ringed seal blubber samples were collected by hunters in the communities of Sachs Harbour, Resolute Bay, Arviat and Nain between 2011 and 2015 and analyzed for 68 (out of 75) PCNs by GC-HRMS. Geometric mean concentrations of ΣPCNs ranged from 0.50 to 5.6 ng/g l.w. across the 4 sampling locations, with the highest levels recorded in seals from Sachs Harbour and Resolute Bay in 2011 and 2012. Tetra- and pentachloro-PCN congeners predominated, representing 89-94% of ΣPCN. Mean concentrations declined from 2012 to 2014 except at Nain, Labrador. These mean concentrations are about ten-times higher than for the same locations sampled over the period 2000-2010. The previous PCN data were determined by low-resolution mass spectrometry and were based on fewer congeners which may explain the lowest levels reported. Polychlorinated naphthalenes are more prominent contaminants in Canadian Arctic seals than previously determined based on new results using improved analytical techniques.

TP049 Metal accumulation and histopathological changes in Arctic hares (Lepus arcticus) inhabiting a Deactivated Lead-Zinc Mine in the Canadian High Arctic

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The current study was undertaken to determine post-mining baseline accumulation of selected trace metals, and histopathological alterations in free-living Arctic hares (Lepus arcticus) inhabiting the vicinity of a deactivated lead-Zinc mine located in the North Baffin Island of the Canadian high Arctic. Trace metal analysis included measurement of arsenic, cadmium, iron, lead and zinc in hepatic and renal tissues and soil, and histopathological assessment involved evaluation and scoring the severity of metal-induced hepatic and renal lesions. In general, metal burden in hepatic and renal tissues of hares collected from the vicinity of the mine did not differ markedly relative to that in hares collected from the reference area. Nonetheless, a higher accumulation pattern of cadmium and lead in the hepatic tissues of hares was recorded in the mine area relative to the reference area, although no corresponding hepatic lesions were observed in animals collected from the mine area. Surface soils near the mine area contained significantly higher levels of trace metals (Zinc> Manganese>Lead>Cadmium>Arsenic) compared to that in the reference area. Moreover, soil cadmium levels were found to exhibit a strong correlation with the cadmium burden in renal tissues of hares. Generally, both mine-impacted and reference animals showed similar but varying severities of hepatic and renal lesions at the sublethal level, notably vascular congestion, occasional large hepatocyte nuclei, binucleate hepatocytes, yellow-brown pigmentation in the cytoplasm of hepatocytes, and clustering of hepatocytes. Only hares collected from the mine area with relatively higher tissue burden of lead showed renal edema, and hemorrahage of the capsular surface. This study represents the first assessment of metal-induced histopathological alterations in Arctic hares inhabiting a historical mining area in the high Arctic.

TP050 Climate Change, contaminants, ecotoxicology: interactions in Arctic seabirds at their southern range limits

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Effects of contaminants on Arctic wildlife are occurring against a backdrop of rapid climate change. Contaminants can cause endocrine disruption and associated impacts on reproduction. Because the endocrine system plays a critical role in allowing animals to respond to environmental stress, endocrine disruption could limit the plasticity of wildlife to respond to climate change. In 2016, we examined how the ability of seabirds to respond to changing ice conditions is impacted by a suite of selected legacy and emerging contaminants. We studied thick-billed murres at their southern range limit, where climate change is strongly impacting seabird health. When ice breaks up earlier, adults must fly further and so expend more energy but ultimately gain less food, and young murres grow more slowly. Hormone systems are likely involved in this phenomenon as high levels of stress hormones (corticosterone) and potentially thermoregulatory hormones (thyroid hormones) impel individuals to increase energy expenditure, fly farther and find alternative prey sources. We expected that high levels of contamination would disrupt hormone regulation, decoupling the relationship between hormones and energy expenditure in seabirds. We anticipated that chemical disruption of that relationship may explain why some individuals appear unable to alter their foraging behavior in response to changing ice conditions.

TP051 Organophosphate flame retardants and plasticizers in the High Arctic: Deposition and Transport in Lake Hazen

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Use of organophosphate esters (OPEs) as plasticizers and flame retardants has increased substantially since the restrictions on polybrominated diphenyl ether (PBDE) flame retardants in the early 2000s. OPEs were thought to be a more suitable alternative with lower persistence and decreased bioaccumulation potential. The long range transport potential of OPEs is uncertain. The modeled atmospheric half-life is very short (< 1 day) which would render them unlikely candidates for long range transport. However it has been hypothesized that OPEs adsorb to aerosols, making them less available to oxidation and increase their potential to be transported over long ranges to non-source regions. We chose the Lake Hazen watershed (82 °N) in Quttinirpaaq National Park on northern Ellesmere Island, Canada to investigate the presence of OPEs and the impact of watershed processes on their loadings. At 540 km^2, Lake Hazen is the tenth largest Arctic lake and the world's largest lake north of the Arctic Circle. Sampling over the 260 m water column of Lake Hazen demonstrated an OPE profile dominated by tris(2-butoxyethyl)phosphate (TBEP, 0.89-8.4 ng/L), followed by chlorinated OPEs: tris(2-chloroisopropyl)phosphate (TCPP, 0.55 – 2.2 ng/L), tris(1,3-dichloroisopropyl) phosphate (TDCPP, 0.13-1.4 ng/L), tris(2-chloroethyl)phosphate (TCEP, 0.085 – 2.1 ng/L). These concentrations are an order of magnitude lower than OPEs in the Great Lakes: 12±2.1 ng/L TCPP, 4.0±1.0 ng/L TDCPP, 1.5 ±0.2 ng/L TCEP, and 75±39 ng/L TBEP. OPEs were detected in the glacier-fed rivers flowing into Lake Hazen as well as the major outflow, the Ruggles River. Concentrations of TCPP, TDCPP, triphenylphosphate (TPP), and TBEP were 2 – 10 x higher in the Turnabout River compared to the Ruggles River. This suggests that the glacier-fed rivers are an important source of OPEs into Lake Hazen. Temporal sampling was conducted to evaluate snowmelt inputs and the role of ice cover on OPEs in Lake Hazen. These results demonstrate the long range transport potential of OPEs and their water-borne mobility in the dissolved phase leading to a consistent presence in the Lake Hazen watershed.

TP052 Long range transport of organophosphate flame retardants and plasticizers: deposition in a High Arctic ice cap

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Organophosphate esters (OPEs) have been used as alternatives to brominated flame retardants, plasticizers, and anti-foaming agents for several decades globally. Applications in varied industries including furniture, textiles, electronics, building materials, vehicle and petroleum industries and typically they are incorporated as additives as opposed to chemically bonded to the substrate. This has led to questions regarding environmental fate and transport. There have been increasing reports of detection in indoor and outdoor air, sediment, water, wastewater, and biota. Hypotheses regarding the long range transport potential of OPEs have emerged based on detection in precipitation and air in remote locations as well as monitoring in oceans. These observations are surprising since OPEs are not expected to meet persistence or physical property criteria for long range transport.The purpose of this research was to evaluate the long range transport potential of OPEs by examining their profile in the high Arctic. To this end, we selected an ice cap located on the uninhabited Devon Island spanning 55,000 km^2 in the Canadian Arctic archipelago. It is at extreme northern latitude, 75 °N and the highest point of the ice cap itself where sampling occurred is at an elevation of 2200 m. Here, we obtained a longer ice core with a bottom depth dating 1979 in order to perform a robust and continuous time series spanning several decades. OPEs were detected in every annual layer of ice from 2014 to 1979.In the most recent year analyzed, 2015, the predominant analyte was TCPP (49%), followed by TnBP (14%), TCEP (9.6%), TPP (9.0%), TBEP (7.3%), TEP (5.8%), TDCPP (3.7%). Together these six analytes comprised 98% of the total OPE concentration.Many of the OPEs were correlated with each other ($p< 0.05$). The strongest correlation was TCEP with TnBP (Spearman correlation coefficient R_S 0.90. The annual deposition rate was used to calculate flux in ng/cm^2 so that temporal trends in deposition could be evaluated. The data indicated increasing deposition of most OPEs since the 1990s. The majority of the analytes displayed doubling times of between 3 and 6 years. The doubling time for the chlorinated OPEs, TCEP and TDCPP, were much longer (14 and 21 y, respectively) suggesting that emissions of these compounds may be tapering. This is interesting given that TCEP and TDCPP were replacement flame retardants for polybrominated diphenyl ethers (PBDEs).

TP053 Transport and bioaccumulation of perfluorinated Compounds in an Arctic lake catchment influenced by permafrost disturbance and climate change

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Although perfluorinated alkyl substances (PFAS) and their precursors differ from the legacy persistent organic pollutants (POPs) in the way they bioaccumulate and bind to proteins, they are globally distributed and found in biota from remote areas, such as the Arctic. The goal of this study was to investigate whether global warming, which is affecting Arctic lakes and their catchments, may impact the transport and bioaccumulation of PFAS. The study was conducted at the Cape Bounty Arctic Watershed Observatory on Melville Island (74°55′ N, 109°35′ W), where two adjacent, geologically similar watersheds with two lakes (West and East) are currently undergoing climate-driven changes. PFAS concentrations (C4-C16 PFCAs, C4-C10 PFSA) were determined in archived Arctic char muscle samples (N=190) collected annually from 2008- 2015. Lake and river water and soil samples collected in 2015 (n=50) around the lakes and watersheds were also analysed for PFAS. Although the two paired watersheds and lakes are adjacent, ongoing studies have revealed significant permafrost disturbances affecting the West Lake watershed which has resulted in high turbidity combined with elevated POC and DOC in comparison to East Lake. Preliminary results show the presence of PFAS in all environmental matrices selected, with levels up to 0.7 ng/L for PFBA in lake water and river, although no statistically significant differences were observed between lakes. Preliminary results for ∑PFCAs and ∑PFSAs in char show slightly higher concentrations in West Lake. Body condition factors (CF) were calculated to assess the health of Arctic char in both lakes from 2008-2015 and results show that CF in West Lake char have declined significantly at a rate of 4.5% per year, while increasing 3.3% per year in char from East Lake. Condition factors between 0.7-1 are typical in char of other Arctic Lakes, while < 0.6 for char of West Lake, implies a low food supply and could explain the slightly higher concentration of PFAS detected in Arctic char from West Lake. In the same way, $\delta^{13}C$ in char muscle was consistently more depleted in East Lake than West Lake suggesting that carbon in West Lake arctic char is more of terrestrial origin. Our preliminary results suggest greater inputs of PFASs in West Lake which may be due to mobilization of previous atmospherically deposited PFCAs and PFSAs during the accelerated permafrost disturbances in the West Lake catchment.

TP054 PCBs in the Arctic atmosphere: Determining important driving forces using a global atmospheric transport model

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We present a spatially and temporally resolved global atmospheric polychlorinated biphenyl (PCB) model, driven by meteorological data. The model is skilled at simulating mean atmospheric PCB concentrations and seasonal cycles in the Northern Hemisphere midlatitudes and mean Arctic concentrations. The model does not, however, capture the observed Arctic summer maximum in atmospheric PCBs. Using sensitivity simulations to assess processes within, outside, or transport to the Arctic, we examine the relative influence of climate- and emissions-driven processes on Arctic concentrations and their effect on improving the simulated Arctic seasonal cycle. We find evidence that processes occurring outside the Arctic have a greater influence on Arctic atmospheric PCB levels than processes that occur within the Arctic. Our simulations suggest that re-emissions from sea ice melting or from the Arctic Ocean during summer would have to be unrealistically high in order to capture observed temporal trends of PCBs in the Arctic atmosphere. We conclude that mid-latitude processes are likely to have a greater effect on the Arctic under global change scenarios than re-emissions within the Arctic.

TP055 Community based seawater monitoring for organic contaminants and mercury in the Canadian Arctic

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In Arctic marine ecosystems numerous emerging and legacy organic contaminants and methylmercury (MeHg) are bioaccumulated from seawater to lower food web organisms and may reach elevated levels in top predators, such as seals and polar bears, as well as humans consuming them as part of a traditional diet. To date, most contaminant measurements in Arctic seawater have been ship-based and are therefore limited to open water conditions and have therefore not examined seasonal variability, including effects of ice cover and snow/ice melt. We have established a community based seawater monitoring program to examine contaminants with varying physical/chemical properties including perfluorinated alkyl substances (PFASs), brominated flame retardants (BFRs) current use pesticides (CUPs), organophosporus triesters (OPEs), and MeHg. Sampling is being carried out in May under ice and in August in open water conditions in Barrow Strait/Lancaster Sound near Resolute, Nunavut where there are modern laboratory facilities. Collections are being carried out using “active” methods (Niskin samplers at 5 depths of the ~100 m water column and large volume sampling through a XAD resin column/ filter) and “passive” methods (polyethylene membranes (PEs) deployed for ~5 weeks). All analyses were carried out in clean laboratories at the Canada Centre for inland Waters in Burlington, ON and Lohmann lab at University of Rhode Island. Analyses of PFASs show that PFOS concentrations have declined steadily since 2008 and are now non-detect (< 1 pg/L). However, PFOA concentrations are higher in 2011-2015 than in 2005-2010 and are associated with partial ice cover and meltwater inputs. OPEs analyses showed lower levels of 9 OPEs (< 0.01-1.5 ng/L) compared to ship-based sampling in the Arctic (14-36 ng/L) (Jantunen 2014). Seawater MeHg concentrations were comparable to those reported for 2004-05 (range ~20-100 pg/L) and were higher in surface waters in May than in August, likely due to losses in the open water season via photodegradation. Results for BFRs and CUPs will also be presented, including comparison of polybrominated diphenyl ethers (PBDEs; a class of flame retardant) using “passive” and “active” methods. Overall, results to date highlight the need to include examination of temporal trends that consider seasonal variability in sampling designs and monitoring programs.

TP056 Investigation of Mercury Toxicity in Landlocked Arctic char in High Arctic Lakes

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In the Canadian Arctic, mercury (Hg) concentrations in the tissues of non-anadromous (“landlocked”) Arctic char are elevated with ~30% of the sampled populations exceeding toxicity thresholds. In 2011, 2012, and 2015 we collected tissues (liver, muscle, brain) from Arctic char (n=167) from four lakes (Small, North, 9-Mile, and Amituk) on Cornwallis Island, Nunavut. The lakes sampled span a gradient of Hg contamination, allowing for the comparison of biological endpoints in char with low Hg concentrations to char with high Hg concentrations. The objectives of this research were to (1) measure total Hg and Hg speciation in Arctic char livers, brains and subcellular components, (2) determine correlations between Hg concentrations and biomarkers of oxidative stress in livers and brains, and (3) assess potential histological changes in livers and brains in relation to Hg exposure. Results thus far show a wide range in total Hg concentrations in Arctic char among lakes (0.05 to 2.5 parts-per-million wet weight in muscle), with about 31 % of individuals exceeding a toxicity threshold of 0.5 parts-per-million wet weight. Methylmercury was the predominate form of total Hg in liver (~80 %), where concentrations were two to three times higher than in muscle (to a maximum concentration of 6.5 parts-per-million wet weight). Total Hg was predominately found in the sensitive subcellular pools (mitochondria, microsomes and lysosomes, and heat-denatured proteins, including enzymes) of low- and high-Hg char, suggesting that increasing Hg exposure does not result in an activation of detoxification mechanisms in the liver. As a result, a significant fraction of Hg may exit the liver and reach the brain, as concentrations in the two organs were similar. To determine if Hg concentrations affect Arctic char, we measured several biomarkers of oxidative stress. Results indicate a positive correlation between total Hg and levels of lipid peroxidation in the livers of Arctic char. This link may explain histological changes in the livers of fish from the most contaminated lake. This research goes beyond documenting Hg concentrations in fish and will provide critical knowledge concerning fish health status.

TP057 Selenium and mercury levels in sub-Arctic versus Arctic prey fish in eastern Canadian Arctic marine ecosystems

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Indirect effects of climate change in the Arctic include altered predator-prey relationships due to shifting habitat ranges of prey fish species. Studies on predator diets suggest that sub-Arctic prey species, such as capelin (Mallotus villosus) and sand lance (Ammodytes spp.), are replacing Arctic cod (Boreogadus saida) as the main prey item, far mostly within the low to mid-Arctic regions. These invaders may have different contaminant levels than similar trophic position residents due to seasonal migrations from the sub-Arctic into Arctic marine systems. Within Arctic marine food webs, mercury (Hg) levels have been increasing in some biota in recent decades. Current levels may put some top predators at risk of adverse biological effects. We previously found from samples collected in 2012-2014 that sub-Arctic invaders actually tended to have lower Hg levels than Arctic resident prey fish. Nonetheless, as selenium (Se) may offer protective effects against Hg toxicity, it has been suggested that Se levels and Se:Hg molar ratios should be considered in evaluating the risks from Hg exposures. We determined the Se levels in muscle tissues of various species of Arctic and sub-Arctic prey fish and the possible contribution of Se as a protective nutrient by calculating the Se-to-Hg molar ratios. We collected Arctic fish and invertebrates (sculpin (Scorpaeniformes), northern shrimp (Pandalus borealis), amphipods (Amphipoda) and Arctic cod) and sub-Arctic fish species (sand lance and capelin) from three differentially-invaded regions in the eastern Canadian Arctic. There were no significant differences in Se levels and Se:Hg molar ratios within species from different regions. All species had similar Se levels, except for sand lance with twice as much Se (1.1 ± 0.2 µg/g) compared to other species ($p < 0.001$). Se levels are generally regulated in body tissues, and thus the differences may be more likely related to species-specific regulation on internal Se levels relative to variation in environmental exposures to Se. There were significant differences in Se/Hg molar ratios among species ($p< 0.001$), except between capelin-sand lance and sculpin-northern shrimp. The highest ratios were found in sand lance (122 ± 15) and the lowest in northern shrimp (6.9 ± 0.4). Nonetheless, the Se/Hg molar ratios were above one for every fish, suggesting protective levels of Se against Hg toxicity for consumption of both Arctic and sub-Arctic forage fish by piscivorous predators.

TP059 Assessment of coliform and faecal coliform bacteria in lake water samples collected from Larsemann Hills area over East Antarctica

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Antarctica is the largest pristine wilderness in the world. Nevertheless, Antarctic waters are being polluted due to anthroprogenic activities caused by various research activities. In Antarctica, coliforms are generated by two sources: local vertebrate populations and human activities. The Larsemann Hills is an ice-free area of approximately 50 km2, located halfway between the Vestfold Hills and the Amery Ice Shelf on the south-eastern coast of Prydz Bay, Princess Elizabeth Land, East Antarctica (69º30′S, 76º19′58″E). The ice-free area consists of two major peninsulas (Stornes and Brooknes), four minor peninsulas, and approximately 130 islands. The Larsemann Hills area contains more than 150 lakes at different Islands and Peninsulas. There are four stations in the area including Bharati Indian Research Station which is located between Thala Fjord & Quilty bay, east of Stornes Peninsula in Antarctica at 69° 24.41' S, 76° 11.72' E approximately at 35 m above sea level.Coliform bacteria are not pathogens themselves, but their presence indicates the possibility of finding pathogens. In contrast, fecal coliform bacteria such as Escherichia coli are found in feces, and their presence in drinking water indicates fecal contamination. E. coli can also be a pathogen itself, so if E. coli is found in water there is a good chance that other pathogens are present. In the present study, eleven lake water samples were analyzed to study coliform and fecal coliform bacteria. Out of six lake water samples collected during 34th Indian Scientific Expedition to Antarctica (ISEA) four samples were found to be contaminated with coliform bacteria, however, Faecal coliform were absent when tested with selective media. MPN coliform were found to be present in two samples (Brookness peninsula (69°22'49.5"S, 76°17'21.2"E) and Mcloed Island (69°22'04.69"S, 76°09' 00.79"E) out of five samples collected during 35th ISEA. Faceal coliform were absent in all samples. Presence of MPN coliform indicates that the degree of pollution and sanitary quality of lake water. Coliform contaminations are found in the lakes which are in the vicinity of those areas which are regularly accessible (L-3, L-1D, L-7A, Murk water). and However, there are other factors which may lead to coliform contamination such as migration of microbes through birds and transport of food items from ship to research station.

Arctic Spill Science – Fate, Effects and Response

TP060 Quantifying the effect of biodegradation on oil toxicity using the PETROTOX model

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Although rare, the accidental release of crude oil during processes such as extraction or transport does sometimes occur, and to effectively and properly respond to these incidents, one must understand the oil's fate and effects in the environment. Knowing the rate and extent of biodegradation of spilled oil is critical to fully understand its fate and potential effects in the environment. Many degradation studies simply use the non-descript measurement of total petroleum hydrocarbons (TPH) to model degradation, which can be useful in understanding what is happening overall, but can misrepresent the toxicological consequences of this degradation. Individual hydrocarbon components which make up petroleum substances have specific and different physiochemical properties which cause them to behave differently in the environment, both in terms of toxicity and degradation. The spreadsheet PETROTOX model developed by Concawe calculates the toxicity of complex petroleum products to aquatic organisms using the substance's detailed chemical composition. This three-phase model simulates the distribution of each structure in the water, air, or oil-phase, then calculates toxicity based on the predicted aqueous concentrations, using the target lipid model, assuming additivity. To simulate expected water concentrations following effective dispersion, relatively small amounts (15ppm) of oil were mixed with water in closed respirometer flasks. To allow for thorough toxicological comparisons of the oil at various stages of weathering, detailed chemical analysis (GC-FID) was performed at four time points (0, 5, 10, 28 days) over the course of 28 days. The present study evaluates differences in toxicity and degradation of crude oil as it weathers over time.

TP062 Effects of oil spill responses on key Arctic zooplankton species

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Increased shipping and oil and gas activities in the Arctic increase the risk of an oil spill. Oil compounds can have toxic impact on Arctic marine ecosystems, but impacts from response technologies on ice associated ecology have not been studied extensively. The copepod Calanus glacialis is a key species in the Arctic marine ecosystem. It plays a central role in energy transfer between primary producers and higher trophic levels of the Arctic food chain. It is therefore relevant to study potential consequences of an oil spill on this ecological important species. As a part of a large joint industry initiative (www.arcticresponsetechnology.org) a first of its kind mesocosm experiment was executed in an Arctic fjord of the Island of Svalbard. Effects of natural attenuation of the oil, in-situ burning and chemical dispersion were studied on grazing, egg production and hatching of the Arctic copepod Calanus glacialis. Eight mesocosms with open top and bottom were deployed in the sea ice in Van Mijenfjorden, Svalbard, in February 2015. Two replicates were used for all treatments. After application, surface ice was allowed to re-establish. Water was collected from the top 2 cm water column in March and just before sea ice break up in May, and was used in two 14-day incubation experiments with C. glacialis collected in Isfjorden. Copepods were fed during the experiment and eggs and pellets were quantified daily. Egg hatching was determined in the beginning and end of the experiment. There was no significant effect of the oil spill treatments on average cumulated specific pellet production or egg hatching success. However in May, the average cumulated specific egg production was significantly higher in the oil-dispersant mixture treatment compared to the control from day 2 (+ 169 %) until the end of the experiment (+ 41 %). To correlate observed effects and toxicity, and to examine potential pollutant transfer, the chemical content of incubation water, exposed females and produced eggs was further analysed for chemical residue. These results will be presented on the SETAC 2016 conference.

TP063 Long-term resilience in polar cod exposed to dispersed oil and burned oil residue

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Scientifically sound information on the efficiency of oil spill response (OSR) actions to reduce environmental impacts is essential for net environmental benefit analysis. The use of chemical dispersants as an OSR is debated, as dispersants are known to significantly increase the oil concentration in the water column for a restricted time. In situ burning of spilled oil may be an alternative OSR to dispersant. However, after successful in situ burning of spilled oil, approximately 10% of the oil slick will remain in the environment as burned oil residue. The main aim of the present study was to assess potential long-term effects on polar cod (Boreogadus saida), an Arctic key species, after an acute exposure to dispersed crude oil or in situ burned oil residue. Wild caught polar cod (N=240) were individually tagged prior to being exposed for 48 hours to clean seawater (control), an environmentally realistic concentration of either mechanically dispersed oil, chemically dispersed oil or burned oil residue (N=60 per treatment). At the end of the exposure period all fish were transferred to a common rearing tank in order to study potential long-term effects on survival, growth (specific growth rate, condition factor) and reproductive investment (gonadosomatic index, gonadal histology) throughout a six months resilience period. The preliminary results show that the total hydrocarbon concentration in the water of the MDO and CDO treatment was 10-20 ppm. Dispersed oil induced a transient short-term reduction in SGR in the first week post-exposure. Over the course of the first 4-month post-exposure, the SGR In the MDO And CDO was compensated and no significant differences between exposure treatments and controls were observed at the following time points. Furthermore, no treatment-dependent mortality has been identified. Histological analyses for reproduction impairment analyses are ongoing. Ultimately, the produced data will be used in the development of the Net Environmental Benefit Analysis (NEBA) tool and in population effect modeling.

TP064 Matrix models for Arctic Calanus species: using experimental toxicity data for oil components to assess population level effects

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When assessing population level effects of oil spills, data are required on both the life history parameters of species and toxicity end-points. We constructed age-structured matrix models for Arctic copepods to predict the impact of potential oil spills at the population level. Two matrix models were defined, representing the Arctic shelf species Calanus glacialis, spawning in waters all around the Arctic shelf and C. hyperboreus, the Arctic oceanic species connected to deep-sea, with a 2x2 and a 4x4 matrix model, respectively, which refer to a 2-year and a 4-year life-cycle. The post-breeding models included egg production and mortality rates as life-history parameters. Relevant Arctic conditions were taken into account by defining a winter diapause period, where no mortality is assumed to take place. Toxicity information on exposure to oil and oil-components was collected for Calanus species. It appeared that the life-history parameter with the highest elasticity in the model was the adult survival, meaning that changes therein have most impact on the output, i.e. population size. This is because the adult class produces offspring. Published data on mortality rates in field and laboratory studies appeared too high to maintain viable populations. Therefore, we modified these parameter values such that modeled populations became viable. Adequate toxicity information on oil and oil components for Calanus species appeared to be scarce, and we therefore selected pyrene as a model compound. However, reported end points for pyrene were not directly applicable in the matrix model, and we developed new approaches to derive a dose-response relationship from the available data. Based on the generic matrix models, simulation models using day-to-day calculation steps were developed to test the effects of various oil spill scenarios at the population level, taking into account a range of exposure concentrations and exposure durations. The results show that the calculated population effects are very different, when based on different types of effect concentrations (NOEC, LOEC and LC_{50}). We argue that simple matrix models are very useful to assess the potential impacts of oil spills at the ecological relevant population level, but that scarcity of information limits an accurate quantitative assessment. A more realistic estimate of natural survival rates and the toxic effects thereupon will allow for a better assessment of population level consequences of an oil spill.

TP065 Produced water exposure causes cardiac toxicity in early life stages of cold-water fish

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Emissions of produced water (PW) to the marine environment in the North Atlantic and Barents Sea are regulated with the overall aim of producing no harmful environmental effects. PW contains variable amounts of polycyclic aromatic hydrocarbons (PAHs), which have the potential to cause severe developmental alterations during the embryonic stage in fish. In the present study, we exposed Atlantic cod (Gadus morhua) and haddock (Melanogrammus aeglefinus) to PW during embryonic development. These species are widely distributed in the North Atlantic, as far north as the western coast of Svalbard. We exposed embryos of both species to three concentrations of produced water for 4 days during organogenesis in an attempt to survey the possibility of PW to cause cardiac toxicity and associated developmental effects on larvae. Thereafter we transferred them to clean seawater where they developed until hatching. No significant effects were observed for survival and hatching success compared to controls. Concentration-dependent bradycardia (reduced heart rate) was observed during both embryonic and larvae stages for both species. Craniofacial and spinal deformations as well as yolk sac oedemas were observed in larvae treated with high PW concentrations during embryogenesis. There is a need to identify the PW components that are causing the observed effects, and if concentrations of these components can exceed the effect threshold in actual PW discharges.

TP066 Effects of produced water components on growth, development and reproduction in Arctic pelagic copepods

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Increasing ice cap melting is opening up new areas in the Arcticto human activities such as oil exploration. Oil drilling involves the release of large quantities of hydrocarbons into the marine environment, notably as produced water and oil droplets. The PWC-Arctic project endeavors to assess the potential environmental impact of oil exploration in the High North by quantifying the uptake and excretion kinetics of produced water components and oil droplets and their effects on growth, development and reproduction of the Arctic copepods Calanus glacialis and C. hyperboreus. These calanoids are key zooplankton species with high lipid content which makes them prone to uptake of lipophilic oil components. Particular focus is on bioaccumulation kinetics, oil component transfer, effects on metabolism, growth, fertility and fecundity of various development stages. Experimental findings include slower excretion of heavier polyaromatic hydrocarbons (PAHs) from copepodid C5 C. glacialis than predicted by a single compartment model while physiological effects of exposure appear to be transitory. The parameterized data collected in this project will provide direct input to numerical models aimed at predicting the impact of produced water on the marine environment in the Arctic.

TP067 Uptake and elimination kinetics for polycyclic aromatic hydrocarbons in Arctic lipid rich copepods

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Oil exploration and production has recently moved further north towards the Arctic, increasing the potential for exposure of Arctic organisms to polycyclic aromatic hydrocarbons (PAHs) during regular production as constituents of produced water (PW) and from accidental oil spills. Hence, understanding the toxicokinetics of PAHs in Arctic species is increasingly important for environmental risk assessments of regular discharges and damage assessment following accidental spills. The calanoid copepods Calanus finmarchicus, C. glacialis and C. hyperboreus are the dominating zooplankton species in the North Atlantic Ocean and the Barents Sea. They store excess energy by accumulating large volumes of lipids during their late development stages before molting into adults. Previous studies have shown that exposure to lipophilic water pollutants such as PAHs results in an accumulation of these components in Calanus species. Traditionally the uptake and depuration kinetics are modelled using one-compartment toxicokinetics. However, previous experimental data on C. finmarchicus strongly indicate that elimination of PAHs is much slower than expected based on calculations by state-of-the-art one-compartment toxicokinetic models such as OMEGA. In the present study, the lipid rich developmental stage copepodite V (CV) of the Arctic Calanus was exposed to a filtered dispersion of alkane oil spiked with 11 PAHs for 120 h at 1 °C. The log Kow of the PAHs ranged between 3.3 for naphthalene and 5.78 for chrysene. A 120 h depuration period in clean seawater followed the exposure period, and samples for body burden analyses were taken at regular intervals during both the exposure and the depuration periods. Models developed to fit to the uptake data were used to predict depuration kinetics for individual compounds. Several of the PAHs, especially the heavier fluoranthene, pyrene and chrysene, showed approximately 100 % retention after the 120 h depuration period, substantial deviations from the predictions by the one-compartment models. The slow depuration of PAHs in lipid rich Arctic copepods demonstrated in this experiment indicates that stored PW components may be more available for transfer to progeny, as well as having a higher potential for transfer to higher trophic levels than previously anticipated.

Use of Freshwater Mollusk Toxicity Data for Improved Conservation of Water and Sediment Quality

TP068 Investigating the impacts of water quality on freshwater mussels

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Freshwater mussels (family: Unionidae) are amongst the most endangered organisms in North America, with over 70% of native unionid species listed as endangered, threatened, or as a species of special concern. Pollution is cited as one of the major factors in the decline of unionids. Recent laboratory toxicity tests indicate that freshwater mussels, particularly in the juvenile life-stage, are amongst the most sensitive aquatic organisms to ammonia. In addition, studies have also shown that concentrations of ammonia can be elevated in pore water (i.e. the interstitial water between particles of sediment) relative to overlying surface water. With juvenile mussels constantly being exposed to pore water via direct contact and pedal feeding, resource managers may be missing a key piece of information by focusing only on concentrations of contaminants in surface water. In 2013 and 2014, U.S. Fish and Wildlife Service biologists collected pore water and overlying surface water at sites in Michigan, Wisconsin and Minnesota to examine any potential correlations between surface water quality and pore water quality, and water quality and mussel abundance. The overarching objectives of the project were to aid decision-making and provide resource managers with additional data and tools (e.g., by enhancing a Mussel Threats Geospatial Database) with respect to mussel reintroduction and restoration efforts. We will present a summary of our methods, results, conclusions and any relevant recommendations that may contribute to future management and conservation of freshwater mussels.

TP069 Acute and chronic toxicity of aluminum to a unionid mussel (fatmucket, Lampsilis siliquoidea) and an amphipod (Hyalella azteca)

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The U.S. Environmental Protection Agency (USEPA) is updating the ambient water quality criteria (WQC) for aluminum (Al). Recent studies have indicated that freshwater mussels are sensitive to some metals (e.g., copper and nickel), ammonia, and major ions (e.g., chloride, potassium, and sulfate). However, little is known about the sensitivity of mussels to Al. Previous studies have demonstrated that Al toxicity to aquatic organisms is influenced by hardness and pH. A pH of 6 favors the presence of all Al hydroxide species and maximizes their solubility, presenting a worst-case condition. The objective of this study was to evaluate acute and chronic toxicity of total Al to a unionid mussel (fatmucket, Lampsilis siliquoidea) and a commonly tested benthic invertebrate (amphipod, Hyalella azteca) at pH 6 in diluted well water (hardness 100 mg/L as $CaCO_3$). The amphipod was one of the most sensitive species in the USEPA chronic Al dataset. The acute (4-day) and chronic (28-day) exposures with mussels and amphipods were conducted in a proportional flow-through diluter with a pH controller to maintain a constant pH of 6.0. Both species survived >95% in all treatments over the acute exposures. Acute EC50 of >6200 µg total Al/L for the mussel and amphipod was greater than the EC50s for other species in the USEPA dataset. Control survival was 100% for mussels and 95% for amphipods at the end of chronic exposures. Chronic EC20s based on dry weight was 163 µg total Al/L for the mussel and 409 µg total Al/L for the amphipod. For both species, the EC20s for biomass were slightly (< 4 to 6%) higher than the EC20s for dry weight. Results indicate that the mussel was more sensitive than the amphipod in the chronic Al exposures. When including the mussel data in the USEPA chronic Al dataset, the mussel would be the fourth most sensitive species with the amphipod being the fifth most sensitive species.

TP070 Acute and chronic toxicity of nickel or zinc to a mussel (Lampsilis siliquoidea) and an amphipod (Hyalella azteca) in water-only exposures

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The USEPA is reviewing the protectiveness of its national ambient water quality criteria (WQC) for nickel and zinc. Previous studies have indicated that freshwater mussels and the amphipod Hyalella azteca are sensitive to nickel and zinc in acute or chronic exposures. However, survival, growth, or reproduction of the tested organisms may not have been optimal in these previous studies. The methods for conducting chronic toxicity tests with mussels and H. azteca have been recently refined, resulting in improved control growth for the mussels and amphipods, improved reproduction for amphipods, and the ability to conduct longer-term (12-week) tests with mussels. The objectives of this study were to evaluate acute and chronic toxicity of nickel or zinc to the mussel Lampsilis siliquoidea and amphipod H. azteca in water-only exposures, and to evaluate the influence of test duration on the toxicity of nickel and zinc to mussels in chronic exposures. Results from both acute and chronic tests reinforced the sensitivity of L. siliquoidea and H azteca to nickel and zinc. In particular, the acute 50% effect concentration [EC50] of L. siliquoidea exposed to nickel was less than half of the final acute value of 940 µg/L in the current WQC at a hardness of 100 mg/L. Similarly, the chronic EC20s for H azteca were >4-fold lower than the chronic WQC of 52 µg/L for nickel and >2-fold lower than chronic WQC of 120 µg/L for zinc. Chronic EC20 for L. siliquoidea was >3-fold lower than the chronic WQC for zinc. Refined chronic test conditions did not appear to change the 42-day sensitivity of H. azteca to either zinc or nickel, relative to previous studies. Increasing the duration of exposures from 4 to 12 weeks with L. siliquoidea did not change EC20s for nickel but decreased the EC20s for zinc by about 50%. The results indicated that refined chronic test conditions did not substantially change the sensitivity of L. siliquoidea or H. azteca to nickel or zinc; however, including the toxicity data from this study in a revision to the WQC would likely lower the WQC for nickel and zinc.

TP071 Maternal metal transfer in the introduced apple snail Pomacea sp. and consequences for their use in pollution biomonitoring

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Two species of the apple snail, Pomacea maculata and P. canaliculata, have recently been introduced in Louisiana marshes. The spread of these invasive species is bound to have a variety of ecological consequences. However, their presence can have a beneficial consequence as well if Pomacea snails can serve as biomonitors for metals such as Pb, Cd and Cu in their environment. One issue that may influence their usefulness in biomonitoring is the maternal transfer of metals. Such transfer would facilitate biomonitoring; it is generally much easier to find the snails' eggs than the snails themselves, as the brightly colored egg clutches are laid above the water level. Insight into the maternal metal transfer will also assist with assessing the ecotoxicological consequences of metal pollution in freshwater environments. The maternal transfer could have effects on the developing young, as has been observed in least killifish studies in our lab. Moreover, maternal transfer of metals can serve as a means of metal elimination, as was seen in previous studies with the crustacean Daphnia magna. To our best knowledge, the occurrence and consequences of metal transfer in apple snails have not been studied. We are currently conducting laboratory experiments to determine effects of long-term, chronic exposure on the reproductive success of Pomacea exposed to metal concentrations. Subsequent work will then address the metal transfer and the consequences on reproductive endpoints such as clutch size, hatching time and hatching success. Exposures of adult Pomacea snails to Pb, Cd or Cu are being conducted with snails collected from the drainage ditches of Patterson, LA. These exposed snails are then transferred to clean water and allowed to lay eggs. One subset of each egg clutch will be analyzed for metal levels while the remainder is allowed to hatch while being monitored for toxicity effects.

TP072 Metal accumulation and biomarkers in a Neotropical freshwater clam for monitoring water bodies at coal mining areas: a case study in Southern Brazil

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Coal mining is one of the highest potentially impactful industry activities and can cause several alterations in the environment, representing an example of a source of metal mixtures in the environment. In this context, the present study focused on the investigation of metal bioaccumulation and sub-lethal effects promoted in the Neotropical bivalve Anodontites trapesialis, after 96 h of confinement in a stream located near a coal mining, aiming to evaluate the efficiency of a set of end-points as tools for monitoring these areas. Bivalves were maintained in cages for 96 h at two sites located upstream (Up1 and Up2) and two sites downstream (Dw1 and Dw2) from the mining area. At each experimental site physical and chemical variables were measured in the water, and samples of water and sediment were collected for metal determination. Mantle, gills, digestive gland, muscle (foot) and hemolymph were collected for metal (Al, Fe, Mn and Zn) bioaccumulation analyses. The following biomarkers were measured in the gills, mantle and digestive gland of the bivalves: total antioxidant capacity, lipid peroxidation, protein carbonylation, glutathione S-transferase activity, metallothionein content and acetylcholinesterase activity. The results showed the influence of coal mining area on the stream water as alterations in some physical and chemical variables were observed at the two sites located downstream. The bioaccumulation of Fe and Al occurred in gills and hemolymph of animals caged at downstream sites. As expected, A. trapesialis caged at Dw sites showed more alterations in the biomarkers analyzed, however animals from Dw1 and Dw2 showed a different pattern in those alterations. In the gills, the occurrence of oxidative damage (lipid peroxidation and protein carbonylation) was observed only in animals caged at Dw2, in spite of the similar metal bioaccumulation in this tissue in animals caged at both Dw sites. In the mantle, lipid peroxidation was demonstrated only in A. trapesialis caged at Dw1, while MT content and TAC alterations were observed in bivalves from Dw2. In the digestive gland no alterations were observed in the biomarkers analyzed. Thus, the Al and Fe accumulation and oxidative damages in A. trapesialis tissues could be efficient tools for monitoring the environment quality of these particular mine.

Measuring and Estimating Bioavailability: Linking Exposures to Effects for Improved Ecological Risk Assessment

TP073 Measuring bioavailable mercury in process wastewater and ambient water: not all mercury is the same

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Regulating bioavailable forms of trace elements is being accepted by a growing number of water resource agencies. In the US, a significant event to promote regulating bioavailable forms of trace elements was the issuance of USEPA's dissolved metals policy (1993). For bioaccumulative trace elements (Hg, Se), the goal of minimizing bioacculation potential is to regulate the most bioavailable species. Despite extensive knowledge on the biogeochemistry and bioaccumulation potential of Hg, most resource agencies regulate total Hg in wastewater samples, with no consideration given to known bioavailable forms (MeHg and inorganic reactive Hg). This study provided preliminary results of Hg speciation in wastewater, ambient, and mixing zone samples. Effluent samples were collected from treated FGD (flue gas desulfurization) wastewater (at four coal-fired power plants) and ambient Ohio River samples. Samples were analyzed for total Hg, dissolved Hg, MeHg, and acid-soluble inorganic Hg. For wastewater samples, the proportion of total Hg as bioavailable Hg ranged from 27 to 100% (median = 32%). For ambient samples, levels of total Hg were low (all less than 3 ng/L), so estimates of bioavailable Hg had considerable uncertainty. Concentrations of total Hg and MeHg in fish tissue samples using fish collected near the four power plants were low (< 0.3 mg/kg wet wt.), thus the Hg discharged from power plant wastewaters had seemingly minimal bioaccumlation potential. The regulation of bioavailable forms of total Hg could prevent the imposition of costly source controls, and is supported by scientific understanding of Hg cycling and fate.

TP074 Quantitative AOP-based predictions for two aromatase inhibitors – evaluating the influence of bioaccumulation on prediction accuracy

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The adverse outcome pathway (AOP) framework can be used to support the use of mechanistic toxicology data as a basis for risk assessment. For certain risk contexts this includes defining quantitative linkages between the molecular initiating event (MIE) and subsequent key events (KEs) within an AOP. One AOP for which strong, quantitative linkages have been established is aromatase inhibition leading to reproductive dysfunction in fish. A series of computational models have been linked to develop a quantitative AOP (Q-AOP). A measure of aromatase inhibition is used as the model input to estimate circulating plasma estradiol (E2) concentration and resultant circulating plasma vitellogenin (VTG) concentration. To evaluate model predictions, two aromatase inhibitors, letrozole and epoxiconazole, were selected based on their relative aromatase inhibition potency in USEPA ToxCast assays. Reproductively mature female fathead minnows (Pimephales promelas) were exposed to varying concentrations of either letrozole (0, 0.5, 7.5, 25, 75, 250 µg/L) or epoxiconazole (0, 8, 25, 80, 250, 800 µg/L) in 24h flow through exposures. One additional consideration for model predictions was bioaccumulation of exposure chemicals and resultant circulating plasma concentration. To identify this, plasma from exposed minnows was extracted by supported liquid extraction (SLE) and concentrations of letrozole or epoxiconazole determined by LC-MS/MS. Plasma bioaccumulation factors (BAF_{plasma}) varied across concentration ranges with lower exposure concentrations leading to greater bioaccumulation. Letrozole (logP = 2.22) BAF_{plasma} ranged 0.173-0.25, indicating rapid metabolism or excretion of the compound, while epoxiconazole (logP = 3.44) BAF_{plasma} ranged 34.4-66.9. Exposure tank concentrations and calculated plasma concentrations were used as model inputs. Model estimates using both inputs will be compared with experimental measurements to highlight potential refinement of Q-AOP predictions. The contents of this abstract neither constitute nor necessarily reflect USEPA policy.

TP075 Bioavailability prediction of estrogenic contaminants in marine sediments

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The frequent input of organic contaminants in marine environment through industrial and urban effluents can be responsible for a pseudo-equilibrium state between aqueous phase and sediments that can directly affect the bioavailability of these compounds in the system. In this work, we have invstigated chemical methods: "E"value and extraction agents as butanol and β-HPCD, to predict the bioavailability of triclosan (TCS), bisphenol A (BPA) and 17α ethynylestradiol (EE2) in marine sediments in a 60-day experiment followed by aqueous phase analysis by GC-MS/MS after solvent exchange and derivatization procedure. The bioavailability study based on "E" value model involves isotopic exchangeability of these compounds and shows lower percentages (< 20%) for all compounds individually and observed in different periods of interaction. For TCS this exchangeability occurs until 7 days of experiment. After this period, TCS shows be strongly complexed with the matrix. This was also evident when data for butanol and β-HPCD were accessed, with an extraction of 80% just on the first days of experiment. For BPA and EE2, the fraction considered exchangeable was lower (< 13%) and the extraction intensity was slightly higher for butanol treatment. According with these results the bioavailability order considering the tested sediment was: TCS>BPA>EE2 although TCS was bioaccessible just in the first days of interaction. In this way, obtained results can be important to help the comprehension of the complex behaviour pathways of these contaminants in marine environment, being an useful precursor test for bioassays and contributing for estimate the risk assessment in this system.

TP076 Building improved in vitro exposure assessment capability: Towards the development and implementation of enhanced QIVIVE tools

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Recently the NRC published a series of reports outlining a strategy for conducting risk assessment in the 21st century, a key emphasis of which is working towards an improved mechanistic understanding of toxicity pathways. Due to the large numbers of chemicals used in commerce, however, substantive challenges exist associated with testing all endpoints and chemicals in an efficient and inexpensive. To address this challenge, recent activity aimed at assessing the effects of a large number of chemicals screened through a suite of in vitro assays, and commonly referred to as high throughput screening, has gained increasing interest. The underlying hypothesis is that toxicological response is driven by interactions between chemicals and biomolecular targets. The goal of high throughput screening is, thus, to focus on a multiple target matrix approach, rather than a single target. The matrix contains an expanded number of potential targets, whose chemical interactions may be characterized by in silico models, biochemical assays, cell-based in vitro assays, and nonmammalian animal models. Data derived from high throughput in vitro screening can thus provide guidance in assessing potential toxicological hazards relevant to both HRA and ERA. Combining high throughput in vitro data with mechanistic insight regarding exposure dose in vivo, however, is proving to represent a substantive challenge. Nonetheless, it has long been understood that chemical toxicity arises from an interaction between a chemical and a site of action that leads to perturbation of a biological function. This is best assessed based on a dose-response relationship, where the dose/concentration delivered to the organism via respiration, diet, intravenous, or dermal, is used as a surrogate for the concentration at the site of toxicological action. Consequently, it is important to emphasize that it is ‘the dose that makes the poison”. In this presentation a number of key challenges associated with ensuring accurate translation of data between in vitro and in vivo systems are highlighted.

Systems Modeling Approaches for Ecotoxicology to Link Molecular Responses to Ecosystem Effects

TP077 Development of a Mathematical Model for Fecundity in Mummichog (Fundulus heteroclitus)

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Ranging from the Atlantic coast of Florida to the Maritime Provinces of Canada, the mummichog or Atlantic killifish (Fundulus heteroclitus) is an important model organism for understanding the effects of pollutants in estuarine and marine ecosystems. In particular, the toxicological effects of stressors on fecundity are central to understanding ecological impacts. We developed a mathematical model to predict mummichog fecundity based on a model developed for fathead minnows (Pimephales promelas). In the fathead minnow model, the concentration of a fish egg yolk precursor protein, vitellogenin (VTG) is a key driver and important predictor of oocyte growth and spawning events. However unlike fathead minnows, mummichogs exhibit non-overlapping cycles of oogenesis, which is associated classically with the lunar cycle. We adapted the fathead minnow model to mummichogby fitting empirical spawning data (clutch sizes and spawning intervals) from mummichog in paired mating experiments and from the literature. We then evaluated the model by predicting independent data that included group spawning design experiments. Preliminary results suggest that the modeling assumptions used for fathead minnows may need to be revisited to improve the mummichog fecundity model. Factors that may be important include: female size, variation of the proportion of non-spawning mature females through the reproductive season, and the strength of lunar periodicity displayed by populations within the species range, where Northern-most populations exhibit low periodicity. We are also evaluating the effects of social interactions (group size) on spawning success. By considering how models can accommodate variation in fish species reproductive strategies, we can more easily develop other fish species-specific fecundity models to predict the ecological impacts of stressors.

Challenges in Testing and Monitoring of Micro- and Nano-Plastic Effects and Environmental Distribution

TP078 Development of analytical procedure for microplastic analysis in biological tissues: application to Mytilus edulis from the Atlantic coast

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Since the middle of the last century, several million tons of plastics have been produced. Whilst the societal benefits of plastic are far-reaching, this valuable commodity is the subject of increasing environmental concern as 10% of produced plastics is estimated to end up in the ocean. Once in the environment, macro debris undergo mechanical, chemical and biological actions. All these degradation processes lead to their fragmentation into microplastics (MPs) which accumulate in the environment. Many different procedures were used for MP quantification and characterization in environmental and biological samples. However, no simple protocol allowing the routine monitoring of MPs is nowadays available. The aim of this study was to develop a preparation procedure to finally apply it for the monitoring of MP in mussels (Mytilus edulis) from the Atlantic coast (Pays de la Loire, France). The digestion, filtration and analysis steps were optimized comparing different solvents (HNO_3 (65%), H_2O_2 (30%), NaOH (30%), HNO_3/HCl (37%) (3:1, v/v), HNO_3/H_2O_2 (3:1, v/v), HNO_3/$HClO_4$ (70%) (4:1, v/v)), filters (cellulose nitrate (5 µm and 12 µm), polycarbonate (0.2 µm), aluminum oxide filter (2 µm), fiberglass and cellulose acetate (1.2 µm)) and analysis modes (reflection, transmission) using µFTIR. Finally, a simple protocol minimizing the time-consuming operations and the sources of errors was validated with spiked samples and presented 70 to 100% of recovery depending of the MP nature (polyethylene, polypropylene, polyvinyl chloride). In a second part, this protocol was applied to wild and cultivated mussels collected at three sites on the Atlantic coast. The results showed few MP of acrylonitrile butadiene styrene (ABS) by individuals. The seasonal and intersite variabilities were discussed.

TP079 Extraction and Analysis of 10-500 µm Sized Microplastic in Wastewater

M. Simon, N.v. Alst, Aalborg Univ; D.A. Stephansen, Aalborg Univ / Civil Engineering; J. Vollertsen, Aalborg Univ

Wastewater is an important source of microplastic. However, rather few studies exist on microplastic in raw and treated wastewater especially when it comes to small sizes. In this work we present a method for sample preparation of raw wastewater and treated wastewater and the following analysis using a micro-FTIR imaging system with a focal plane array detector (FPA). For analysis of particles below 80 µm we use transmission of IR through a sample while larger particles are analyzed by reflection. For each identified particle its size, form and material is determined. The focus point of this presentation is the methodology of sample preparation, pitfalls and possibilities as well as lessons to be learned on sample preparation for subsequent micro-FTIR imaging. Wastewater is a challenging matrix for analyzing microplastic particles, which is further complicated by the small size range targeted. The sample preparation and analysis hence is extensive. For raw wastewater, 1 L of sample is pre-treated by wet-sieving on a 500 µm sieve. Due to the strong adherence of plastic particles to other particles, a surfactant is added prior to sieving. A sub-sample is treated with cellulase, upon which the sample is oxidized by adding hydrogen peroxide and a catalyst. The initial enzymatic breakdown of cellulose is required because cellulose fibers (from toilet paper) otherwise limit FTIR analysis as they tend to coat the plastic particles. The oxidized samples are wet sieved into 80-500 µm and ≤80 µm intervals. The particles are removed from the sieves and gathered into demineralized water by ultrasonic treatment and filtered separately through a 10 µm stainless steel mesh. Particles are then collected in ethanol. A sub-sample of the ethanol is transferred to a transmission window (≤80 µm particles) or a reflection window (80-500 µm particles). For treated wastewater, varying sample volumes are collected as the content of suspended matter differs much between treatment plants. Known amounts of wastewater are filtered on-site onto 10 µm stainless steel filters until they clog. Filters are then treated enzymatic and oxidative. The recovery rate of the method is tested with 100 µm polystyrene beads and found to be around 80%. Additionally, the results from a field study is presented where 10 of the largest Danish wastewater treatment plants have been sampled and the efficiency of the treatment plants to remove microplastic particles in the size range of 10-500 µm evaluated.

TP080 Extraction, Identification and Quantification of 10-500 µm Microplastics in Soils and Road Dust Using Micro-FTIR Imaging

N.v. Alst, M. Simon, Aalborg Univ; D.A. Stephansen, Aalborg Univ / Civil Engineering; J. Vollertsen, Aalborg Univ

The occurrence of microplastic in agricultural soils is poorly known. This study focuses on the occurrence of microplastic in the size range 10-500 µm in soils that received wastewater sludge as fertilizer. It presents the method of sample preparation and presents data from a field study, but centers on the identification of microplastic using micro-FTIR imaging in terms of the methodology, pitfalls and possibilities of this technology. In the first step of analysis for soil and road dust, a sample is suspended into a surfactant solution, slightly agitated and wet sieved into 4 size fractions of 10-40, 40-80, 80-200 and 200-500 µm. Each sample is transferred to a straight glass funnel with stopcock, for gravimetrical separation of the inorganic fraction and a 1.7 g/cm3 zinc chloride ($ZnCl_2$) solution is added. The sample is agitated for approximately 20 minutes by aeration from below. The aeration is turned off, and organic material sticking to the side of the glass is flushed into the bulk liquid with $ZnCl_2$ solution. The column is left to stand for 2 hours, and approx. 200 mL of the top liquid column is drained through a side port. The $ZnCl_2$ solution is resupplied and the floatation sequence is repeated twice. The mixed liquid obtained from the separation is filtered over a 10 µm steel filter, which is then oxidized in H_2O_2 enhanced by a catalyst. The oxidized sample is again filtered over a 10 µm steel filter, and the particles suspended in ethanol. For particles ≤80 µm, a subsample of the ethanol is deposited on an IR transmission window mounted in compression with a clear aperture of 10 mm. For particles >80 µm, a subsample is transferred to an IR reflective MirrIR microscope slide on which a steel washer is mounted, also restricting the area to a diameter of 10 mm. The sample amount is chosen so the window is suitably populated for micro-FTIR analysis. The ethanol is evaporated and the sample analyzed using an Agilent micro-FTIR Imaging system, which is equipped with a 128x128 pixel Mercury Cadmium Telluride (MCT) Focal Plane Array (FPA) microscope detector. Particles ≤80 µm are analyzed in transmission mode with a 25x or 15x objective, and particles >80 µm analyzed by reflection using a 4x objective. With the 128x128 MCT FPA detector, this gives individual pixel sizes of 3.3x3.3, 5.5x5.5 and 20.1x20.1 µm and a field of view of 422x422, 704x704 and 2640x2640 µm respectively. For all identified particles its size, form and material are recorded.

TP081 How we may get it wrong using standard test guidelines to test for effects of plastic particles

A. Palmqvist, Roskilde Univ / Dept of Science and Environment; F. Khan, K. Syberg, Roskilde Univ / Dept of Science and Environment

Plastics have become ubiquitous and seemingly unavoidable contaminants in the environment. A major part of it, at least when measured as abundance rather than mass, is in the form of micro- and probably nano-plastic particles. Whereas larger sized plastic litter has obvious impacts on e.g., exposed reptiles, birds and mammals, particles on the nano- to micro scale is likely to affect smaller sized animals that play important ecological roles for example by forming the basis of food-webs or contributing to the degradation of organic matter and cycling of nutrients. As such it is necessary that we explore the hazard of nano- and micro-sized plastic particles to organisms inhabiting all types of environments. Ecological effects of chemicals is typically tested under controlled conditions in the laboratory, in order to avoid or be able to consider confounding factors, using tests as prescribed by standard test guidelines (e.g., OECD, US-EPA). Such tests are optimized for testing chemicals in solution, and in many cases to test for chemicals that may be directly taken up from the surrounding medium over external surfaces (e.g., skin and gills). Few standard tests are suitable to test for effects of contaminants that exert their effect through ingestion of the contaminant. For contaminants on particulate form ingestion is precisely a likely prerequisite for exerting and detecting maximum effects. In this presentation we will provide examples from both aquatic and terrestrial tests highlighting how exposure conditions prescribed by standard test guidelines may give a false impression of the inherent hazard of small scale plastic particles. We will also provide some suggestions on how to overcome some of these obstacles with manageable adjustments of standard tests.

TP082 Pristine Micro- and Nano-Plastics Readily Form Microorg-Agglomerations in a Lab-Simulated Marine Environment

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Researchers have shown that small sized (nano- / micro-) plastic fragments are present throughout the oceans, yet their ultimate fate is still unclear. Numerous laboratory studies, conducted with pristine plastic particles, have investigated their effects on marine organisms; however, the environmental relevance of such experiments are in debate. In the natural marine environment, microorganisms and exopolymeric substances (EPS) are associated with plastics and this association may alter the behaviour and effects of plastic particles. The flocculation of plastic particles, EPS and microorganisms (termed here microorg-agglomerates [MAGs]) should be investigated in order to improve our understanding of the behaviour and effects of plastics in the marine environment. To address this in the present study, MAGs were created in vitro using pristine polystyrene particles which were then incubated with natural seawater on roller tables; a range of plastic sizes was employed (50 nm – 10 mm) in separate experiments. Additionally, the mechanisms of MAG cohesion were examined using several microscopy techniques and the microbial community assessed using MiSeq sequencing. The behaviour of plastic fragments in an aquatic environment is partly dependent on size. Smaller particles have a greater colloidal stability, suggesting that they remain in suspension, separate from other plastic pieces. However, our research shows that plastics ≥50 nm flocculate to form MAGs up to 1 mm in size within 7 days, under laboratory conditions. In addition to plastics, MAGs were shown to comprise of particulate matter and a rich community of microorganisms found entrained within an amorphous matrix of EPS, which acts as a cohesive agent bonding the MAGs. The presence of EPS and particulate matter within MAGs may alter the buoyant density of these agglomerates, influencing their sinking velocity and position in the water column. Our findings reveal that plastics can form into micro- and millimetre-scale MAGs, potentially influencing their fate. In addition, the formation of MAGs may result in a modification of the behaviour of plastics as their buoyancy and colloidal stability is altered. Changes in the rich microbial community associated with plastic agglomerates may also play an important role in influencing the fate of chemical pollutants sorbed onto or incorporated into the plastics, in the uptake of pathogens by consumers, and more broadly upon their impacts to higher trophic levels.

Leveraging Technologies Between Mammalian and Ecological Toxicology to Advance Risk Assessment

TP083 Dose-level selection and exposure refinement in mammalian toxicology

N. Burden, NC3Rs

The benefits of incorporating exposure considerations within the safety assessment process are being increasingly recognised. This includes improving understanding of internal exposures and increasing the human relevance of doses that are used in mandatory animal toxicity studies. Toxicokinetic (TK) analyses are already a routine component of the safety assessment of pharmaceutical candidates. Exposure data for chemicals is currently largely limited to estimations and measurements of applied external concentrations. There are therefore opportunities to increase the use of TK data to inform the design and utility of toxicity studies for agrochemical and industrial chemicals. Such data could be used to inform dose selection for subsequent toxicity studies and ensure they are relevant to predicted human internal exposure levels. This would obviate the need for the traditional maximum tolerated dose approach, and the dosing of unnecessarily high test concentrations which result in development of toxicities in animals that bear no real-life relevance. Effects observed when high doses are tested often trigger further toxicity studies and investigations into mode of action which may not be necessary. The advent of sophisticated microsampling techniques also brings advantages, as circulating blood levels can be correlated to observed toxicities within the same animal. This can not only increase understanding of dose-response relationships but also decreases the need for groups of satellite animals dosed solely for TK analyses. This type of exposure-driven approach is applicable to both human health and environmental safety assessments, and supports the generation of data that is directly applicable for making risk, rather than purely hazard-based, assessment decisions.

TP084 Adverse outcome pathway as a unifying collaborative framework to tackle challenging ecological problems: a case study using behavior in larval fish

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The concepts behind adverse outcome pathways (AOPs) are not necessarily new, but the coordination of a general framework, terminology, and resources is a recent development in the past decade. We found these qualities of the AOP to be an effective collaborative platform to unite multidisciplinary scientists to focus on challenging ecological problems, to form hypotheses and direct research. In our work, we focus on behavior, which is a challenging endpoint to incorporate into ecological risk assessment because it is difficult to infer how subtle changes in behavior translate to ecological metrics (such as population abundance trajectories), and it is often difficult to setup appropriate behavioral assays in the laboratory (especially for species not typically maintained in the lab). But behavior of organisms, if measured appropriately in laboratory assays, can provide much integrated insight into the physiological status of the individual, with implications for key demographic parameters such as individual survival and growth rates. We use the AOP framework to unite several different technologies and approaches: behavioral assays, gene expression, metabolomics, individual based models, and risk analyses to predict the effect of contaminants across levels of biological organization and infer population responses to quantify risk of exposure to certain chemicals. By using the AOP framework in a systematic way, we collect data on multiple species and facilitate cross-species comparisons. We also collaborate with other agencies and institutions that do similar work on different species – which assists our cross-species comparisons. Our aim is to determine what information can be collected on model species that will be informative for behavioral impairments and population responses on other species. We also wish to determine if molecular information can substitute for behavior assays related to foraging success and learning, as such assays are labor intensive and challenging to do on non-model species and molecular level information is relatively easier to collect. The AOP framework has been immensely helpful for organizing, focusing and propelling this research forward.

TP085 Mammalian toxicokinetics and alternative approaches to toxicity testing

A. Lowit, USEPA / Office of Pesticide Programs

Toxicokinetic information has great value in dose selection, evaluation adverse outcome pathways, cross-taxa evaluation/extrapolation, lifestage susceptibility, and reducing or replacing in vivo animal toxicity studies. This presentation will provide a discussion of the importance and value of having in vivo and in vitro toxicokinetic information in assessing risk to pesticides. Case study examples will be used to elucidate the principles of using toxicokinetic information in helping to assess toxicity testing and risk assessment. The talk will describe the use of in vitro toxicokinetic studies to compare across species and to assess variability. Finally, the presentation will highlight progress being made on by the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) and the NIEHS under the National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) in the area of developing alternative approaches for acute toxicity.

TP086 Global probabilistic environmental hazard assessment of antihistamines

L.A. Kristofco, Baylor Univ / Dept of Environmental Science; B.W. Brooks, Baylor Univ / Dept of Environmental Science

Global population concentration is increasingly occurring in megacities, where access to medicines is increasing more rapidly than waste management infrastructure is implemented. Antihistamines (AHs) represent a common class of pharmaceuticals, and have been shown to make their way through the sewage treatment process. As streams and many aquatic systems are increasingly dominated or dependent on effluent discharge of differential quality, chronic low dose exposures to non-target species must be considered. However, hazard and risks of AHs to non-target species are poorly understood. Here, we present a global assessment of AHs in surface waters and effluents. Antihistaminergic active pharmaceutical ingredients (APIs) were identified, literature occurrence and ecotoxicology data on the identified APIs was collated, therapeutic hazard values (THVs) for each API were calculated, and environmental exposure distributions (EEDs) of the APIs were compared to ecotoxicity thresholds, drug specific and cumulative THVs to estimate hazards in surface waters and effluents. 60 unique AH APIs were identified using the DrugBank and MaPPFAST databases. Literature searches on the occurrence of the identified AHs in environmental matrices was completed and, after consolidation returned < 100 unique occurrence publications in environmental matrices in Asia-Pacific, Latin America, Europe, and North America. However, the majority of surface water (63%) and effluent (85%) observations were from Europe and North America, which highlights relatively limited information from many regions, including developing countries and rapidly urbanizing areas in Africa, Latin America and Asia. Less than 10% of all observations were for estuarine or marine ecosystems, though the majority of human populations reside close to coastal ecosystems. Though diphenhydramine was most commonly reported, cimetidine, cinnarizine, loratadine, and ranitidine represented other frequently detected AHs. EED 5th and 95th centiles for all AHs in surface water, influent and effluent were 2, 6, and 5 ng/L and 212, 4287, and 1308 ng/L, respectively. Though some ecotoxicology publications for antihistaminergic APIs were identified, few of these examined mixtures or mammalian read across predictions. This study identifies the utility of global assessments of AHs and other pharmaceuticals to identify regions where environmental monitoring, assessment and management efforts appear necessary.

TP087 From Zero to Validated Adverse Outcome Pathway (AOP) with Genomics Guiding the Way

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Genomics investigations served as the foundation for discovery of a critical adverse outcome pathway (AOP) with relevance to both ecological and human health. The work represents a suite of studies that established the molecular, mechanistic and systems-level impacts of the most abundantly distributed class of munitions contaminants, the nitrotoluenes. Historical toxicological investigations of nitrotoluenes identified lethargy, weight loss and muscle wasting as general adverse outcomes (AOs) of exposure. In order to identify the source(s) of these AOs, global-transcriptomics expression investigations of nitrotouene exposures were conducted to identify the genes and pathways affected. A recurring theme of inhibited expression for transcripts involved in lipid & systemic cellular-energy metabolism pathways were observed in multiple species including rats, mice, birds, and fish in response to various nitrotoluenes. Effects on these pathways were confirmed in proteomics and lipidomics investigations where lipid catabolism was impaired leading to fatty acid accumulation in the liver in conjunction with muscle wasting and overall weight loss. An additional recurring omics response to nitrotoluenes was significant enrichment of peroxisome proliferator-activated receptor (PPAR) signaling pathways where expression of component genes were strongly inhibited. PPARs represent key transcriptional regulators of pathways controlling systemic energy metabolism, where the PPARα isoform controls various facets of lipid transport and metabolism. We then tested the effects of nitrotoluenes on PPARα signaling in human in vitro nuclear receptor inhibition assays confirming that various nitrotoluene structures caused significant signaling inhibition. Given the knowledge above, we developed a hypothetical AOP establishing PPARα binding / inhibition as the molecular initiating event (MIE) for a series of key events leading the adverse outcome (AO) of overall starvation-like weight loss. The connection between impaired PPARα signaling and the AO was validated in PPARα knockout (K/O) mice where the PPARα K/O eliminated the AO resulting from nitrotoluene exposures. Computational docking assays support the putative MIE indicating probable binding of nitrotoluenes to the PPARα active site, a hypothesis presently being tested in PPARα competitive-binding assays. Overall, this work demonstrates the power of omics techniques for leading systems-level development of AOPs.

TP088 Extrapolation Strategies for Ecological Risk Assessment with Inhalation Risk Assessment for Cetaceans as an Example

A.B. Rosenstein, Environmental Risk Expertise; T.K. Collier, Independent Consultant / Oceans and Human Health Program

Scaling (also called the allometric approach) is a theoretical approach used to predict toxicity in species for which toxicity data are lacking – it incorporates various biological, anatomical and physiological differences between species. In mammals, this approach characterizes how patterns of size, anatomy, physiology, and lifespan can affect toxicological endpoints. However, for many mammalian species, knowledge of the exact anatomical and physiological values and their variation is limited or non-existent. An additional uncertainty is that the allometric approach does not take into account differences in metabolism between species. How do current wildlife toxicity extrapolation strategies incorporate scaling factors and how much does lack of data affect the end results? We will present a summary of extrapolation approaches that have been used in ecological risk assessments, and will discuss promising methods currently being developed to take into account metabolic, physiological, and other species differences. As a case study, we recently extrapolated laboratory animal inhalation toxic effect levels to marine mammals (cetaceans) by scaling, using body mass and lung volume. Other physiological and metabolic differences between terrestrial mammals and cetaceans, some of which exist because cetaceans live and feed in deep water versus on land, are not well understood. Examples include differences in: lung physiology and nasal filtration; toxicant uptake because of gas pressures at depth versus on land; and metabolism. These factors were not incorporated into the extrapolations we have conducted so far. We will address the following question: what was the potential effect on our final exposure and risk estimates of omitting these factors?

Developments and Barriers in the Use of Bioavailability for Contaminated Soil and Sediment Human Health Risk Assessments

TP089 Applying site specific adjustments for arsenic: Bioavailability, bioaccessibility, and regulations

Y. Lowney, Alloy, LLC / Health Sciences

The available literature establishes that the bioavailability of arsenic in soil is reduced relative to the soluble forms of arsenic that serve as the basis for the regulatory toxicity criterion (cancer slope factor). This includes studies conducted for soils from individual sites or broader efforts that include soils with several sources of arsenic and soil types. Investigations have also included both in vivo studies using different animals and in vitro methods. The USEPA has determined that this body of evidence supports a default assumption of a 60% RBA for arsenic in soils, to be used when site-specific analyses are not conducted. This presentation will describe the evidence and process of reduced bioavailability of arsenic from soils, and also describe existing and emerging efforts to develop less expensive, “in vitro” methods to estimate arsenic RBA on a site-specific basis. Similar to the in vivo work, the investigations to develop an in vitro method for arsenic have included soils from individual sites, several sites, and more recently, a meta-analysis of in vitro data across several studies. The focus of this discussion will be on the bioavailability of arsenic in soils to human receptors, and describe the incorporation of arsenic bioavailability considerations into the human health risk assessment process.

TP090 Assessing the In Vivo Oral Bioavailability of Polycyclic Aromatic Hydrocarbons from Soil: Approaches and Challenges

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The literature includes several animal models and experimental approaches for assessment of the oral bioavailability of polycyclic aromatic hydrocarbons (PAHs) from soil. Each of these models and approaches has limitations that often include critical untested assumptions and experimental compromises. The metabolism of PAHs, within the GI tract as well as the systemic circulation, and substantial biliary excretion, pose significant challenges in developing sound oral bioavailability assessment methods. While there is almost universal agreement that a simple, cost-effective, reliable in vitro oral bioavailability method will be the most practical way to obtain site-specific relative bioavailability information, validation of candidate in vitro methods requires in vivo data that are scientifically sound to serve as benchmarks. For in vivo estimation of oral bioavailability of PAHs, there are a variety of animal models to consider, as well as endpoints such as measurement of parent compound and/or metabolites in blood, urine, feces, and tissues. Each of these has a rationale, but also technical limitations. Recent studies incorporating a radiolabeled benzo(a)pyrene tracer in weathered soil have allowed evaluation of bioavailability assessment methods for PAH concentrations in soil down to 1 ppm or less, which is the range in which remediation decisions need to be made for many PAH-contaminated sites. These studies have also revealed potential problems in determining an orally bioavailable concentration of PAHs in soil when soil concentration data are derived using standard analytical extraction procedures (e.g., EPA Method 3550C). This presentation highlights the progress that has been made in developing a consensus in vivo model system for PAH oral bioavailability.

TP091 Bioaccessibility of Native Polycyclic Aromatic Hydrocarbons and Derivatives in a Fuel Soot Using an in vitro Gastrointestinal Model

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Soot particles disperse in air and settle on soil and environmental surfaces. Gastrointestinal exposure to PAHs and PAH derivatives in soot can occur by ingestion of soil or environmental particles via hand-to-mouth activities (especially for children) and by swallowing expectorant containing inhaled atmospheric particles. We measured the apparent bioaccessibility (B_{app}) of eleven PAHs, one nitro-PAH and four oxo-PAH derivatives present in a fuel soot using an in vitro three-stage GI model. The model included silicone sheet as an absorptive sink in the small intestinal stage. The soot-borne contaminants were regarded to exist in either a labile or a nonlabile state. Silicone absorption was expected to promote soot desorption by steepening the concentration gradient across the soot-fluid interface, mimicking the effect of absorption by the small intestinal epithelium. Consistent with this expectation, silicone significantly increased B_{app} and reduced the labile fraction remaining on the soot residue after digestion. We also studied the effects of changing physiological conditions in the digestive tract associated with food ingestion. The B_{app} was independent of gastric pH and addition of dietary proteins and carbohydrates, but it increased with bile acids concentration, small intestinal pH (5.00–7.35), and addition of dietary lipids. Rising small intestinal pH increased B_{app} by favoring mass transfer from nonlabile state to labile state, from labile state to digestive fluid, and from digestive fluid to silicone sink. Under fed conditions, B_{app} increased with inclusion of lipids by favoring mass transfer from nonlabile state to labile state, and from labile state to bile acid/lipid micelles in the digestive fluid. The extrapolated maximum possible (limiting) bioaccessibility (B_{lim}) ranged from ~30 to ~65% among the PAHs and PAH derivatives. Evidently a large fraction of these contaminants in fuel soot cannot be mobilized, a result with implications for risk assessment. In addition, B_{lim} was independent of molecular size or K_{ow}, suggesting that a major determinant of bioaccessibility is not contaminant hydrophobicity, but the distribution of chemical between nonlabile and labile states in the original solid. Lastly, the results indicate significant variability in soot PAH bioaccessibility within the range of physiological conditions experienced by humans, and suggest that bioaccessibility will increase with co-consumption of food, especially food high in fat.

TP092 Design and Implementation of In vitro Dermal Absorption Studies of PAHs from Impacted Soils for Application in Human Health Risk Assessment

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Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous in the environment from both natural and anthropogenic sources. Currently, seven of these PAH compounds are listed as probable human carcinogens with benzo(a)pyrene (BaP) serving as the surrogate compound for evaluating mechanisms of carcinogenicity and relative carcinogenic potency in humans. For the first time, the EPA is proposing a dermal cancer potency slope factor in the pending toxicity assessment for BaP (EPA, 2013). The preliminary dermal slope factor, which is based on occurrence of skin tumors at the site of contact in mice exposed to BaP in solvent, may drive future remediation decisions if approved as currently proposed. Since most human exposures to PAHs occur by contact with impacted soil rather than being dissolved in solvent, the proposed dermal slope factor is likely to overestimate the risk of skin tumors in humans. Reduced bioavailability of PAHs in soil compared to PAHs in solvent is documented in the literature. The source(s) of PAHs to the soil and sequestration of PAHs in the soil matrix over time is believed to account for reductions in their observed bioavailability. Numerous in vitro dermal bioavailability studies have been performed evaluating dermal absorption of PAHs from soil using static or flow-through diffusion cells consistent with Organization for Economic Cooperation and Development (OECD) guideline 428 for testing skin absorption of chemicals. Design of an in vitro dermal absorption study using diffusion cells should include the following elements: Skin model and preparation; Receiving fluid; Experimental design; Soil test article preparation; and Flux calculation. Considerations including human versus animal skin, PAH binding in the stratum corneum, metabolic activity of the skin, effects of receiving fluid, sample collection and frequency, study duration, sample preparation and dosing, and control experiments are examples of design elements that will be addressed. Data interpretation and calculations for application in human health risk assessment and establishment of risk-based cleanup goals will also be presented. A case study will be provided evaluating the dermal bioavailability of PAHs in soil impacted by clay target fragments at a skeet range site.

TP093 Predicting and reducing PAH dermal bioavailability from soils with different source materials

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Polycyclic aromatic hydrocarbons (PAHs) are released into soils within different sorption domains (e.g. soot, coal tar or NAPLs) that may alter their bioavailability. Black carbon, such as activated carbon and char have been shown to provide strong sorption domains for PAHs and greatly reduce the freely dissolved concentrations (C_W). Our previous study has discovered strong correlation between soil partitioning coefficients (K_d) and dermal flux simulated by in vitro uptake into pig skin, and that dermal uptake may be well predicted by C_W. The objectives of this study were to 1) investigate the effects of biochar amendment on PAH dermal bioavailability from soils, and 2) use diffusion models to explain and predict dermal uptake by humans from soils. In this study, a number of baseline soils were contaminated with different source materials (soot, coal tar and fuel oil) to achieve a range of PAH concentrations. A subset of the contaminated soils was then treated with 2% biochar to investigate the effect of carbon amendment on PAH dermal exposure from soils. The soil K_d and C_W were determined through aqueous partitioning experiments using polyoxymethylene passive samplers. And the dermal exposure was simulated using in vitro diffusion into pig skin. The soil amendment using 2% biochar was able to reduce PAH dermal uptake by nearly 40% to 90%, through reduction of C_W. However, the effect of biochar varied with different PAH sources: e.g. greater reduction was observed in solvent spiked than soot spiked soils. Strongly correlation between dermal flux and C_W was once again observed, but over a range of C_W across nearly 3 orders of magnitude. Steady state diffusion model derived from Fick's first law was initially used to predict dermal uptake using C_W andsteady statepermeability (Kp). However, the steady state model failed to give adequate prediction (of within 1 log unit deviation) due to the potential disequilibrium condition within skin. Therefore, a kinetic diffusion model derived from Fick's second law involving skin diffusivity (D) and skin/water partitioning coefficient ($K_{S/W}$) is being used to predict the dermal flux, which calls for accurate measurement of D and $K_{S/W}$. $K_{S/W}$ isbeing experimentally determined through skin/ water equilibrium test, and D will be extrapolated using a subset of experimental data (from solvent spiked soils). The kinetic model's performance will be evaluated for the various source materials against measured results.

Environmental Chemistry Perspectives from Around the Globe

TP094 A brief introduction to ChemTHEATRE, the platform to manage the monitoring data of chemicals in environment

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A huge number of monitoring data on persistent organic pollutants (POPs), candidate POPs, pharmaceuticals and personal care products, and heavy metals in various environmental and biological specimens have been reported in scientific journals. However, almost all the valuable data set of the above chemicals are merely stored in simple electronic files, such as text and excel files, by each researcher, and hence they are poorly utilized for other scientists, e.g., to simulate the environmental behavior and fate, and to assess the risk. In the present study, we are developing a platform to deposit and visualize the monitoring data of chemicals in environment, named ChemTHEATRE (Chemical in the THEATRE: Tractable and Heuristic E-Archive for Traceability and Responsible-care Engagement). ChemTHEATRE stores chemical concentrations in environmental specimens with metadata of samples (sampling date and location, organism and its biometry, and so on) and of experimental methods (extraction method, used standards, instruments, etc). This platform enables us to visualize temporal and spatial distribution pattern of registered contaminants, and also to simulate their behavior and fate in the environment. Additionally, using this platform, you may directly reach the specific environmental specimens stored in the environmental specimen bank (es-BANK) at Ehime University, which allows us to obtain some additional data from the same samples, if necessary. At the first step of the platform development, we designed the database structure and registered the monitoring data of dioxins and other organochlorine compounds, brominated flame retardants, and organotin compounds in finless porpoises (Neophocaena phocaenoides) and skipjack tuna (Katsuwonus pelamis). Please visit ChemTHEATRE (http://www.chem-theatre.com/), free to access. We will also appreciate if you would kindly contribute to ChemTHEATRE to register your published monitoring data.

TP095 Effects of Pavement Type (asphalt vs. concrete) on Trace Metals in Road Dust

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Operation of motor vehicles is a major source of environmental contaminants, especially in urban areas. Trace metals are released through abrasive wear between brake pads and rotors; tires and road surfaces. Oil and greases are released from improperly maintained vehicles, and asphalt pavement. To investigate the effects of pavement type on the release of contaminants into the environment, road dust samples were collected on concrete and asphalt sections of Highway 59 in Houston, Texas. Upon arrival at the laboratory, samples were sieved to 250-125 µm, 125-63 µm, and < 63 µm fractions and total environmental-available trace metals were quantified using ICP-MS. Trace metal concentrations increased with decreasing dust size, while trace metal mass decreased with decreasing size fraction. Although < 63 µm particles contained highest trace metal concentration, 250-125 µm particles contributed the greatest overall trace metal mass. Mass weighted average concentrations of < 250 µm size fraction for Co, Cd, Ni, Cu, Zn are 1.8 ppm, 3.1 ppm, 37.3 ppm, 104 ppm, and 185 ppm, respectively for concrete road dust and 2.7 ppm 4.2 ppm, 23.2 ppm, 64.4 ppm, and 265 ppm, respectively, for asphalt road dust. Trace metal concentrations are comparable to previous studies in Houston and other urban areas. The results indicate a significant difference may exist in Cu and Zn concentrations between asphalt and concrete pavement. Study results may be useful for determining road construction materials that contribute less contaminants to urban air and streams.

TP096 The seasonal trends of trace element behaviors in the bottom of Lake Koyama-ike, Japan

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Lake Koyama-ike in Tottori in Japan is a lagoon at present, and it has been a freshwater lake for about 400 years. To improve the water quality, seawater introduction was started in 2012 and as a result the salinity of the lake water drastically changed in the year 2012. The salinity became 14-fold higher than that in 2011. In the present study, 18 trace element concentrations (Li, Al, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Ag, Cd, Sn, Sb, Ba, Pb) determined in sediment, pore-water and bottom water of Lake Koyama-ike in 2013, we compared the trace element levels in the samples between in June and in September. 15 element concentrations (Al, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Ag, Cd, Sn, Sb, Pb) in the sediment were higher in September than in June. It may show that the elution of elements from sediment decreases into bottom water and/or sedimentation and adsorption from water to sediment accelerate. We considered pore-water/ sediment ratio as the elution potential. The elution potential in June was higher than in September. It was reported that Fe and Mn tended to precipitate at pH exceed 8.0. pH values of the bottom water in all stations of Lake Koyama-ike in September were over 8.0. There were 9 elements (Al, Cr, Mn, Co, Ni, Cu, Zn, Cd, Sn) which showed positive correlations ($p < 0.05$) with Fe. From this result, it seemed that Fe and the 9 elements co-precipitated. Next, we assessed the toxicity of the trace elements in the sediment and the bottom water. In the risk assessment using sediment, As, Fe, Mn concentrations exceeded the levels which were concern of the organisms. In the toxicity assessment using the bottom water and pore-water, Zn concentration in the one station exceeded the level of influence on the reproductive function. To understand the bioavailability, therefore, continuous monitoring of the trace elements elution which elute easier from sediment into bottom water is important.

TP097 Relationship between temporal trend of trace element concentrations and grain size in the sediment of Lake Koyama-ike, Tottori

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Lake Koyama-ike in Tottori in Japan is a lagoon at present, and it has been a fresh water lake for about 400 years. To improve the water quality, seawater introduction was started in 2012 and as a result the salinity of the lake water drastically changed in the year 2012. The salinity became 12-fold higher than that in 2011. In trace element concentrations in sediment, 7 elements (Cr, Co, Ni, Cu, As, Cd and Pb) significantly increased from 2011 to2013. There is a report that the concentrations of finer particle size are higher than coarse it. In the present study, Sediments of Lake Koyama-ike were separated into fraction of coarser than silt:CS (> 63 µm) and fraction of finer than silt:FS (?63 µm). 21 trace element concentrations of CS and FS were determined in Lake Koyama-ike. As a result of having compared trace element concentrations of FS with of CS, There were no elements showed significantly higher than FS in CS.

The elements showed significantly higher than CS in FS were Li, Mg, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Rb, Cd, Sn, Cs and Pb in 2011, and Li, Mg, Cr, Mn, Co, Ni, Cu, Zn, Se, Rb, Cs and Pb in 2013. The elements that common significantly higher than CS in FS in 2011 and 2013 were 12 elements (Li, Mg, Cr, Mn, Co, Ni, Cu, Zn, Se, Rb, Cs and Pb). The compositions of the grain size in 2011 and 2013 were 1.2 % in FS and 98.8 % in CS, and 2.4 % in FS and 97.6 % in CS, respectively. Thus, it found that FS fraction in 2013 was 1.2 % higher than in 2011. With regard to the reason why the total metal concentrations in 2011 increased in 2013, we estimated the contribution of the FS fraction to the changes of the trace element concentrations. The trace element which was the highest contribution was Mn 40.7%, following Mn, Fe was 22.1%, Zn was 19.2% and Cu was 19.0%. Contribution rates of Mn, Fe, Cu and Zn were relatively higher than of the others. Next, we investigated chemical forms of Fe and Mn with Eh-pH diagram. As showing in Fig.1, it was assumed that two species $Fe(OH)_3$ and $FeCO_3$ were dominant in the sediment. As showing in Fig.2, it was assumed that three species $MnCO_3$, Mn_3O_4 and MnOOH were dominant in the sediment. Therefore, it was guessed that it was easy environment to elute in Mn. In future study, chemical form analysis and elucidation of the mechanism of concentration changes are needed.

TP098 Risk assessment to lead exposure in Colombian Caribbean coast

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Workers are exposed to lead involuntarily through their contact with lead in their working environment. The work activities developed when cutting, handling and / or melt the metal represents a direct exposure. Many people earn their livelihood by carrying out labor activities in informal work places where they are highly exposed to metal contamination with a low protection and non-environmental health education. Therefore, there are adverse health implications of exposure to high level of Pb because its toxicity may result in damage to the central nervous system, developmental deficiency of red blood cells, cardiovascular system and may have a concomitant influence disorders human fertility in individuals. This research, workers (n = 258) between 18 and 80 years were examined in various trades with more than 5 years in office work involving lead exposure among which are welders, fishermen, repairers batteries, mechanical technician, carpenters , electricians, electronic technician and painters. For this study Pb concentration in blood was determine and using an atomic absorption spectrophotometer with graphite furnace (GF-AAS), Thermo Fisher Scientic iCE Series 3500. Diluting the blood sample in a mixture of Triton X-100, nitric acid, and ammonium hydrogen phosphate $(NH_4)_2HPO_4$ which was used as matrix modifier. Temperatures calcination and atomization optimized were 500Ԩ and 1600 ЂC respectively. Pb concentrations found vary according to labor activity in securities from not detectable (ND) to 72.80 mg /dL Pb. The average concentrations Ђ standard deviation (range) were recycling battery: 11.4 т 21.4 (ND- 72.8), fishermen 11.0 т 11.5 (0.25- 40.8), electricians: 11.37 т 17.8 (1.2 – 51.3), mechanical technician: 10.6 т 4.62 (ND – 59.0), electronic technician: 3.96 т 5.6 (ND – 7.91), painter 3.8 т 4.2 painter (ND – 11.7), welder: 3.7 т 7.3 (ND – 41.2), carpenter: 2.45 т 0, 95 (1.38 – 3.53) and control: 0.83 т 0.90 (ND Ђ_ 2.61). Establishing as a safe level value of 5 mg/dL in adult blood. Recycling battery, fishermen and welders proved trades increased risk to lead exposure, presented greater number of individuals sampled with values> 5 mg / dL. Acknowledgments: Colciencias and Universidad de Cartagena funded grant No. 110765843679.

TP099 Protein expression of gastropod Stramonita haemastoma exposed to antifouling biocide TBT

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Tributyltin (TBT), triphenyltin (TPhT) and other organotins derivatives were widely used as a biocide in antifouling paints in the early 60's to improve efficiency and the mobility of ships. These compounds are known to accumulate along the food chain, acting as an endocrine disruptor in marine organisms, with toxic and adverse effects in many tissues, including vascular system. Due to the formation of a dimorphism of male sex organs (penis and vas deferens) in females, the Stramonita haemastoma is considered as a bioindicator for Tributyltin (TBT). The TBT is considered to be the most toxic substance ever introduced into the marine environment. The proteomics analysis reflects the alteration of protein expression by environmental adaptations, providing a useful framework for the development of new tools for biomonitoring related types of contaminants. The purpose of this study is to determine the changes in protein expression in mollusc gastropod S. haemastoma exposed to tributyltin (TBT). The organisms were collected from Santos estuarine system. The Santos estuarine system are located in the São Paulo State, Southeastern Brazil. This area comprises a major industrial complex, as well as the largest port of Latin America (Porto of Santos). Currently, both harbour and industrial activities combined with the urban contribution represent the main sources of contaminants for coastal and marine ecosystems. Proteins were extracted using phenol / methanol, quantified by Bradford method and fractionated by OFFGEL using strips with pH range from 3 to 10. The method consists into flow protein solutions under a pH gradient gel immobilized through an electric field applied perpendicular to the direction of the flow. Then, the samples were analyzed by SDS – PAGE and identified by nano LC-MS. It is expected to find different proteins between different animals (males, females and hiposex animals).

TP100 Residues of Organochlorine Pesticides: Contamination of Surface Waters in Armenia

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In modern scientific literature, great attention is devoted to integral indicators such as the sum of concentrations of HCH isomers and the sum of concentrations of DDT isomers and metabolites, because their Maximum Allowable Concentrations (MACs) were set considering the amount of the mixtures. In order to determine the degree of pollution in some rivers and reservoirs of theRepublic ofArmenia by persistent organochlorine pesticides water sampling was done. 26 water samples were analyzed and 390 findings obtained. Determination of organochlorine pesticides was carried out by gas chromatography on Shimadzu chromatograph with electron capture detector and a glass capillary column 30 m × 0.32 mm × 0.25 µm (Equity??- 5). As follows from results obtained, water samples contained: DDT (100% samples), Heptachlor (96% samples), HCH isomers (81% samples), Hexachlorbenzene (35% samples), and Aldrin (4% samples). Dieldrin, Endrin, and Mirex were not revealed in analyzed samples. The residual amounts of above-mentioned pesticides varied in the range of: – Aldrin: 0-0.011 µkg/L (MAC = 2 µkg/L); – Hexachlorbenzene: 0.004-0.026 µkg/L (MAC = µkg/L; – HCH (sum of isomers concentrations): 0-0.251 µkg/L (MAC = 2 µkg/L); – Heptachlor: 0.016-0.339 µkg/L (MAC = 50 µkg/L); – DDT (sum of isomers concentrations and metabolites concentrations): 0.108- 2.917 µkg/L (MAC = 2 µkg/L). Levels of Aldrin, HCH, and Heptachlor did not cause any concerns, as they were below the MACs. DDT concentration levels (isomers and metabolites) were dangerously close to MAC, while in some cases (5% samples) the reveled DDT concentrations (isomers and metabolites) exceeded MACs. Undoubtedly, the appearance of pesticides in water is the result of their leaching from the soil and, as a consequence, we might state that the content of these pesticides in soil is much higher. This fact was also confirmed by our previous studies. Identification of DDT metabolites (DDE and DDD) signifies to earlier application of DDT that had already metabolized, while the revealed DDT signifies to "fresh" application of DDT as such. Upon the analysis of study results the following was revealed: – there were no samples with only DDE and DDD residues; – samples with identified DDT, DDE and DDD made 18%; – samples with only DDT residues made 81%. This latter phenomenon indicates to "fresh" application of DDT despite its prohibition

TP101 Shining light on environmental contaminants in sensory organs of pilot whales

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Synchrotron X-ray fluorescence imaging (XFI) is a powerful non-destructive tool for visualizing the distribution of elements of interest in biological specimens down to the subcellular level. Any element (phosphorus and heavier), if concentrated enough, can be detected and the presence of other elements does not decrease its efficacy. The elemental distribution images are built by raster scanning a specimen in the X-ray micrometer-sized beam and collecting the excited X-ray fluorescence signal from each irradiated spot. The remarkably high elemental specificity of this technique lies in the fact, that X-ray fluorescence photons emitted by certain element will have a unique range of energies that cannot be mistaken with any of the other elements. XFI has been successfully applied to answer a broad spectrum of biological questions; however, its application in environmental toxicology is relatively novel. Here we present an application of XFI to investigate the lifetime exposure to various metals/metalloids including mercury in the sensory organs of pilot whales. Previous XFI studies using acutely exposed zebrafish larvae (e.g. Korbas et al. ACS Chem. Biol. 2013, 8: 2256-2263) identified eye and potentially the inner ear as the target sites for methylmercury accumulation. Mercury presence in the sensory cells will likely have detrimental effects on animal vision, hearing and olfaction, which in turn may cause abnormal behavior and decreased survival rate in the wild. The aim of this study was to probe the retina and inner ear from the long-finned pilot whales (Globicephala melas) for the presence of toxic metals and metalloids including mercury. The tissues were sampled from pilot whales from the Faroe Islands in connection with the traditional drive fishery. The acquired material was fixed in formalin, embedded in paraffin and sectioned at 10-micron thickness. Of two adjacent sections, one was stained with H&E to obtain a histological image, while the other, intended for synchrotron XFI was mounted on a metal-free plastic cover slip without any further processing. The 5-micron resolution elemental maps including Ca, Fe, Zn, As, Se and Hg were measured with XFI. To facilitate the identification of the target areas/cells, the elemental distribution maps were then compared with their respective histological images. This study should contribute to better understanding of the extent of wildlife risk associated with long-term contaminants exposure.

TP102 Urinary concentrations of phthalates metabolites in Brazilian children

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Human exposure to phthalates have received considerable attention due to their ubiquitous occurrence and potential adverse health effects. Little is known on exposure of Brazilian population to phthalates. The aim of this study was to establish the baseline concentrations of phthalates metabolites in 300 Brazilian children. Twenty three phthalates metabolites were analyzed in 300 urine samples of 6-14 years old children from five regions in Brazil (southeast, south, central-west, northeast and north) by liquid chromatography-tandem mass spectrometry. Overall, 9 metabolites (mono-ethyl phthalate (mEP), mono-(2-ethyl-5-carboxypentyl) phthalate (mECPP), mono-(3-carboxypropyl) phthalate (mCPP), mono-[(2-carboxymethyl) hexyl] phthalate (mCMHP), mono-isobutyl phthalate (mIBP), mono-n-butyl phthalate (mBP), mono-(2-ethyl-5-hydroxyhexyl) phthalate (mEHHP), mono-(2-ethyl-5-oxohexyl) phthalate (mEOHP) and mono-methyl phthalate (mMP)) were found in at least 95% of the samples with the highest median concentrations of mEP (57.0 ng/mL), mECPP (52.8 ng/mL), mIBP (43.7 ng/mL) and mBP (41.3 ng/mL).

Fate and Effects of Metals: Aquatic Biological Perspectives

TP104 A Combined Laboratory and Field Analysis of the Sulphate Dependence of Selenium Bioaccumulation in Algae

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Sulphate can reduce selenate uptake in algae and aquatic plants, which effectively decreases the amount of dietary selenium available to subsequent trophic levels. However, sulphate dependence is difficult to characterize in field datasets because aqueous sulphate and selenium concentrations often covary. We therefore combined laboratory and field studies to characterize the sulphate dependence of selenium bioaccumulation at the base of the aquatic food web in surface waters where aqueous sulphate and selenium concentrations are correlated. A laboratory study was conducted to model the effect of sulphate on selenium uptake kinetics and steady-state selenium bioaccumulation in algae under controlled conditions. Analysis of uptake kinetics confirmed that tests had achieved steady-state, allowed evaluation of whether the effect of sulphate occur via changes to selenium uptake, elimination, or both, and allowed growth dilution to be quantified so that test results could be more directly related to field data. These results were then combined with field data from coal-mining regions in British Columbia, Canada. This laboratory-to-field integration involved characterizing the combined effect of covarying aqueous selenium and sulphate concentrations, and accounting for differences in algal growth between laboratory cultures and natural streams. The resulting 'hybrid' bioaccumulation model integrated laboratory and field information to model how sulphate concentrations and sulphate:selenium ratios can influence selenium bioaccumulation at the base of aquatic food webs.

TP105 Acute and chronic toxicity of metal mixtures containing cobalt, copper, and nickel to Ceriodaphnia dubia and fathead minnows

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Historically laboratory-based acute and chronic toxicity studies have focused on testing the effects of a single toxicant to an organism. Although a reductionist approach suggests that the conduct and interpretation of single-compound toxicity tests would be the best approach for predicting toxic responses to metals in the environment, "real world" exposures typically consist of simultaneous exposure to multiple metals. Water quality-based regulatory approaches, e.g., USEPA's Ambient Water Quality Criteria and the EU's Predicted No Effect Concentrations, have been established for individual chemicals and do not directly consider potential interactive effects resulting from exposure to metal mixtures. Acute and chronic toxicity tests were conducted to develop an understanding of the toxicity of complex mixtures containing Co, Ni, and Cu. A toxic unit (TU)-based approach where acute or chronic concentrations (LC_{50}s or EC_{10}s) were identified for each metal singly (LC_{50} or EC_{10}s quantified as one TU), followed by exposure to binary and tertiary (Co/Cu/Ni) metal mixtures in equivalent ratios of TUs. Standardized

USEPA acute and chronic testing methodologies were used. Endpoints were assessed to determine whether the mixtures exhibited additive (no difference in toxicity as a mixture), less-than-additive (reduced toxicity as a mixture), or more-than-additive (increased toxicity as a mixture) effects. In acute tests with C. dubia, mixtures generally exhibited a less-than-additive effect, while in the fathead minnow acute test, additive or greater-than-additive effects were observed. In the chronic tests with C. dubia, results indicated either additive or less-than-additive interaction. In the chronic tests with the fathead minnow, with the exception of one of the Co/Ni mixtures (that exhibited a less-than-additive effect), all the mixtures' indicated an additive effect.

TP106 Assessment of mining affected boreal lake sediments as a source of multimetal exposure to the benthic fauna

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Because advanced technology demands vast amounts of metals, there is a growing need for mining activities in many countries. Although mining operations have evolved technologically, metal mining activities have generally long term environmental impacts. In Finland, the soft water boreal lakes are sensitive to ion loading since the buffering capacity is usually low. We assessed ecotoxicological risks of two different mining sites in 11 mining-affected lentic locations and two reference lake sites in Finland, with hypolimnion and sediment field sampling, environmental quality standard comparisons and a chronic toxicity test using a laboratory reared freshwater Oligochaeta Lumbriculus variegatus. This sediment-dwelling blackworm species served as a good representative of the freshwater lake bottom fauna. The national environmental quality standards for water were not exceeded during our sampling time, except for soluble nickel and cadmium concentrations in two sampling sites. Metal concentrations in the sediments were generally greater close to the mining sites. Preliminary results indicate the inhibition of both growth and reproduction of L. variegatus exposed to field-sampled hypolimnion and sediment along the contamination gradient. Our study also brought out the challenges of assessing contaminated sediments together with hypoxic hypolimnion as they are. With our authentic approach the aim was to overcome limitations related to application of standardized biotests to field-collected samples in order to gain a precise case-specific assessment of ecotoxicological risks of the two different mining sites. We will also discuss pros and cons of methodologies besides results indicating deterioration of environmental quality of water bodies downstream the mining sites.

TP107 Bioaccumulation of Heavy Metals by Aquatic Organisms from Historic Mining Operations in Arkansas

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The Rush Mining District along the Buffalo River in Arkansas has a significant history of zinc and lead mining operations. The tails and spoils of these operations deposit heavy amounts of raw ore into streams. One element commonly found in the earth's crust that becomes a minor constituent of the deposition is cadmium. Periphyton samples from Rush Creek and Clabber Creek, two creeks within the Rush Mining District were measured for cadmium as well as two creeks with no history of mining, Spring Creek and Water Creek. Periphyton samples from Rush and Clabber Creek contained mean cadmium concentrations of 436.6±67.3 and 93.38±8.67 µg/kg, respectively. Spring Creek and Water Creek had a mean cadmium concentration of 40.49±3.40 µg/kg and 41.78±3.99 µg/kg within periphyton. The results indicate increased metal concentrations in algal communities from mined areas. As periphyton is the base of the aquatic food chain, it acts as a conduit for movement of cadmium in the food web. The ongoing project will include analysis of bioconcentration within invertebrates.

TP108 Comparison of Cu biouptake by Selenastrum capricornutum in the presence of organic matter from wastewater effluent and stormwater runoff

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Urban streams receive Cu from both treated wastewater and stormwater runoff sources. Both sources have significant quantities of organic matter (OM) which is known to control Cu speciation and bioavailability. The purpose of this study was to experimentally assess and model Cu biouptake to the freshwater algae Selenastrum capricornutum, including both internalization and surface attachment, in the presence of different source and size fractions of OM. Dissolved OM was either left intact or separated into small (0-650 Da), medium (650 Da-50 kDa), or large (50 kDa-0.45 µm) size fractions using tangential flow filtration (TFF). Conditional stability constants of the OM were determined using competitive ligand exchange-solid phase extraction. Metal ligand concentrations were determined from Cu titrations. Conditional stability constants of the whole effluent OM were 0.5 to 1 log unit higher than those of stormwater OM for both hydrophilic and hydrophobic OM (Hydrophilic: Log $K_{Cu\text{-}eff\text{-}OMf}$ = 8.2±0.2, Log $K_{Cu\text{-}storm\text{-}OM}$ = 7.5±0.3; hydrophobic OM: Log $K_{Cu\text{-}eff\text{-}OM}$ = 8.9±0.4, Log $K_{Cu\text{-}storm\text{-}OM}$ = 7.9±0.1). The stability constants of the source OM were all higher than those of algae (Log $K_{M\text{-}total}$ = 6.3 and Log $K_{M\text{-}intra}$ = 6.8). Based on short-term uptake experiments, the results show there was 46% less total Cu uptake in the presence of effluent OM than stormwater OM, while intracellular Cu was about 25% less with effluent. This difference corresponded to the higher conditional stability constants measured for effluent OM than stormwater OM. We also found Cu mainly bound hydrophilic OM (80%) in effluent while it mainly bound hydrophobic OM (77%) in stormwater. Incorporating the conditional stability constants and metal ligand concentrations into an algal uptake model, the Cu uptake could be predicted. As for attachment to algal surfaces, stormwater OM promoted more accumulation of Cu on the algal surface, but this accounted for a small percentage of the total algal Cu content. Furthermore, there was a difference in Cu biouptake and stability constants between effluent and storm size fractions. For effluent OM, the Cu uptake and stability constants across size fractions were similar to the whole effluent. For stormwater OM the Cu stability constants were the lowest for the small size stormwater OM, resulting in more Cu uptake as stormwater OM size decreased. Differentiation between OM sources could lead to better predictions of metal uptake.

TP109 Comparison of fish mercury and selenium in fish otoliths and tissue, can we reconstruct the past?

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The composition of hard tissues is often used to reconstruct conditions in the past. For example tree rings and fish scales can be used to determine ages, growth rates, and other parameters. Fish otoliths grow throughout the life of a fish, with new material being deposited virtually continuously. The mineral matrix of otoliths contains various trace elements and, much like in muscle tissue, the concentrations of these elements is expected to respond to their concentrations in water. The goal of this work was to determine if otoliths could be used to estimate Hg and Se in muscle tissue. We measured Hg and Se in fish otoliths, tissues, and in surface water at seven locations in the Ohio River system. Trace elements in otoliths were measured using LA-ICP-MS, allowing very fine scale transects of metals concentrations and providing a time series of concentrations as the fish grows. It was assumed that the otolith concentrations measured in bone deposited during the last year of the fish's life would be most closely reflective of muscle tissue. There was a significant correlation between tissue and otolith Se, but the correlation was very weak for Hg. For Se, the correlation was significant for 3 of the 10 species with a sample size of 5 or more i.e., bluegill, small channel catfish and freshwater drum, with R^2 values of 0.60, 0.93, 0.71. Thus, for

many species there is also a high degree of variability in the relationship between tissue and otolith concentrations that makes directly relating the two difficult. We developed specie specific regression models that include a variety of water quality parameters in an attempt to account for confounding factors. The inputs tested in the model included various metals, pH, hardness, suspended solids, conductivity, and various nutrients. There was sufficient data to test the model for bluegills, channel catfish, hybrid striped bass, and sauger. For selenium, the best fit models included surface water selenium, and sulfate. For bluegill, channel catfish, hybrid striped bass and sauger the R^2 were 0.83, 0.70, 0.21 and 0.20. The results indicate that predictions of tissue Hg from otoliths is highly uncertain. However, for at least three species the results suggest that otoliths may be useful predicting tissue concentrations. By relating the date of deposition of otolith bone material to Se concentrations, it appears likely that for some fish species historical trends in tissue Se could be quantified and analyzed.

TP110 Comparisons of arsenate and arsenite interactions with periphyton with implications for trophic transfer

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Periphytic biofilms on benthic substrates are important food resources in aquatic ecosystems. These biofilms can also sit at the interface of oxic waters and hypoxic sediments, and as such can be exposed to trace elements in different oxidation states. Arsenic speciation is tied to environmental redox conditions and is also subject to biological alterations. In this study we compared the stability of aqueous arsenate and arsenite solutions under aerated and non-aerated conditions, both in the presence and absence of periphytic biofilms. In the absence of periphyton, As solutions that initially were predominantly arsenite (90% arsenite, 10% arsenate) were slowly oxidized over 7 days resulting in final solutions that were ~65% arsenite and 35% arsenate. Aeration did not strongly affect oxidation rates. In aerated arsenite solutions in the presence of periphyton, solution chemistry changed more dramatically, with rapid oxidation to arsenate and an increasing proportion of organic As forms forming over time. These changes were less dramatic under non-aerated conditions, suggesting that oxidation and methylation reactions were likely performed by organisms that require more oxic conditions. We also compared periphyton enrichment in periphyton exposed to AsV or AsIII (20 ug/L, static renewal, 7 days) and showed similar accumulation patterns of total As (101.3±26.7 mg kg-1 dw and 87.63±22.4 mg kg-1 dw, respectively). The accumulated final concentrations of arsenic in periphyton were 6281- and 6684-fold, higher than the aqueous concentrations they were exposed to. Mayfly (Maccaffertium sp.) larvae that were allowed to feed on AsV or AsIII treated periphyton were similar in their tissue concentrations (13.9 and 14.6 mg kg-1 dw, respectively), resulting in significant biodilution (approximately 10% of their dietary concentrations). The predominant form of As in both of the these biofilm treatments was AsV. Thus, periphyton is an important player in controlling the behavior, speciation and trophic transfer of As in aquatic food webs.

TP111 Dependence of Ecotoxicity of Zinc on Weight of Test Fishes: a Data Exploration and Modeling Based on Biotic Ligand Toxicity Model (BLM)

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Ecotoxicity of certain chemical may be related to weight of the test organism, which will help interpret the variation in observed ecotoxicological effects and improve the ecological risk assessment. Driven by this motivation, the authors selected fatal effect data on fishes by zinc (Zn) to probe whether and how such a relationship exists. Fish test records for Zn in ECOTOXicology database (ECOTOX) were retrieved but only those were retained reporting both the life stage and weight of the organism, and the calcium (Ca^{2+}) concentration of the test solution. Finally a total of 258 data were extracted for adult fish tests with duration varying from 3.6 h to over 1000 h. The data exhibited a significant correlation between test fish wet weights and median lethal concentrations (LC50). The correlation coefficient was -0.23 ($p=2\times10^{-4}$) when all 258 data were used. When limiting the test duration no more than 120 h, the remaining 212 data exhibited a slightly weaker correlation with the correlation coefficient being -0.18 but still statistically significant (p=0.01). A Biotic Ligand Toxicity Model (BLM-TOX) was proposed to model the toxicological effect of Zn, accounting for the competition of Ca^{2+} and Zn^{2+} at gill uptake, and elimination or accumulative detoxification of Zn^{2+}. The BLM-TOX included three fundamental parameters, No Effect Concentration (NEC), Killing Rate (K_+), Eliminating and Accumulating Rate (K_{ga}). Nonetheless, the K_{ga} was further resolved into two parameters, the Maximum Eliminating and Accumulation Rate ($K_{ga,max}$) and a Compensatory Response Time (Beta). Under the BLM-TOX modeling scheme, three of the BLM-TOX parameters, NEC, K_+, $K_{ga,max}$ were considered possibly to be modulated by fish weight through certain linear models. With AIC as the model selector, the best BLM-TOX model was obtained: NEC=0,Beta=190.97 (1/h), K_+=0.12*log(W) (hl/mg), $K_{ga,max}$=47.08*log(W) (1/h) for the 258 dataset; NEC=0,Beta=15.48*log(W)+204.78 (1/h), K_+=0.076*log(W)+0.67 (hl/mg), $K_{ga,max}$=99.92*log(W)+12.65 (1/h) for the 212 dataset. The selected model seemed fitting the observed lethal concentrations (LCs) reasonably well, confirming the influence of test fish weight on the ecotoxicity. Such a step exploring the influence of test fish weight on ecotoxicity is expected to benefit the ecological risk assessment after further refinement and demonstration.

TP112 Development of a Draft Selenium Ambient Water Quality Criteria for the Protection of Aquatic-Dependent Wildlife

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Selenium is found in aquatic ecosystems from the mining of metal ores and phosphates, fly ash from coal-fired power plants, and from leaching of agricultural soils (Presser et al. 2004). At low concentrations, selenium is an essential nutrient. However, selenium slightly above beneficial concentrations can be toxic for wildlife (Ohlendorf 2003). The bioaccumulation of selenium through the aquatic food web can place predatory organisms at risk. Avian species appear to be particularly sensitive to selenium exposure. Population level effects, such as malformations and reproductive impairments, have been observed at environmentally relevant concentrations in aquatic-dependent avian species (Ohlendorf et al. 1986; Hoffman et al. 1988). Therefore, to ensure that aquatic-dependent avian species are protected from exposure to selenium, USEPA is undertaking the task to develop a selenium criterion with the use of the latest scientific literature. The USEPA plans to derive a bird tissue criterion value by assessing food chain bioaccumulation with the use of updated models. Once finalized, this selenium criterion may be used by states and tribes as the basis for adopting water quality standards to protect aquatic-dependent wildlife.

TP113 Effect of pH and hardness on the toxicity of zinc, copper, cadmium, and Nickel to the freshwater diatom Navicula pelliculosa

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Metal effects on algal growth for species other than green algae have not often been investigated. Also, it has been widely known that metal toxicity is highly depending on water chemistry such as pH, hardness, and dissolved organic matter. Here, we investigated the effect of pH and hardness on the toxicity of zinc, copper, cadmium, and nickel to the freshwater diatom, Navicula pelliculosa. The values of pH and hardness of basic medium (modified OECD medium) were 7.0 and 47 mg/L (Ca:Mg ratio is almost 1:1 as molar concentration), respectively. The values of pH were varied to 4 different level: 7.0, 7.4, 7.8, and 8.2 (buffered by MOPS). Hardness were varied to 4 different concentration: 47, 77, 107, and 137 mg/L. Each toxicity test was conducted using 96-well microplates for 72 h, and the algal growth was monitored using a fluorescence microplate reader. Free ion activities in test solutions were calculated using chemical equilibrium speciation software WHAM6. The values of EC10 and

EC50 were determined by concentration-response analysis based on free ion activity. Effects of pH on the toxicity were substantial for copper and not so substantial for zinc, cadmium, and nickel. Effects of hardness on the toxicity were substantial for zinc and nickel and not so substantial for copper and cadmium. The metal bioavailability models were developed using above toxicity data. The model was based on independent effects among pH and hardness according to Deleebeeck et al. (2008, Sci Tot Environ 407, 1901). Model parameters were determined using above toxicity data. Comparisons between experimentally obtained and predicted EC50 and EC10 values showed fairly well agreement (almost all errors were within 2-factors).

TP114 Effects of dietary methylmercury on the dopaminergic system in adult fathead minnows and their offspring

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Mercury (Hg) is a potent neurotoxin which can be globally transported once released into the atmosphere, contaminating even the most pristine aquatic ecosystems. Once deposition occurs, bacteria in the sediment can transform it into methylmercury (MeHg), an organic form capable of bioaccumulation and biomagnification in food webs. As a result, long-lived organisms at the top of the food web are at risk of exposure to MeHg through their diet, which can then be actively transferred from mother to offspring. Exposure during neurodevelopment can lead to serious, irreversible neurological dysfunction, associated with a variety of cognitive and motor abnormalities across species. At even low dietary concentrations associated with regular fish consumptions, exposure can lead to deficits in attention, and hyperactivity. Due to the abundance of confounding variables in human exposure studies, investigations using model organisms which focus on highly conserved biological systems are useful in revealing mechanisms of neurotoxicity. The present study uses one such model organism, Pimephales promelas, to propose a mechanism by which MeHg in the maternal diet may lead to hyperactive behaviors in offspring, and neurochemical changes in adult brain regions. Adult fatheads were exposed to a control diet (0.02 ppm Hg dry weight) or a low diet (0.72 ppm Hg dry weight) for 30 days. Adult brain tissue and embryos from each treatment were collected and analyzed for total Hg, dopamine (DA) concentrations, and monoamine oxidase (MAO) activity. Embryos from treated tanks displayed hyperactivity when compared with controls, as well as significant changes in DA concentrations, seen also in the telencephalon of adult brains. Exposure to MeHg also corresponded with a significant decrease in MAO activity in both eggs and brain tissue. These results suggest alterations to this highly conserved, and tightly regulated neurochemical pathway, due to Hg exposure, may lead to hyperactivity in offspring. Newly hatched larvae were also analyzed for differences in metabolites resulting from exposure to maternally transferred MeHg. Significant changes in abundances of metabolites associated with nervous system injury, and fatty acid metabolism were indicated. Collectively, these results suggest that current environmental exposure scenarios may have negative implications for development and survival of fish exposed to maternally transferred MeHg.

TP115 Effects of embryonic arsenic exposure on killifish's (Fundulus heteroclitus) behavior and nutrient uptake and availability

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Arsenic is found as a contaminant of drinking water, rice, and other crops. Epidemiological studies have shown that embryonic exposure to arsenic can cause changes in behavior and reductions in growth, but the mechanisms for these effects are not well understood. Thus, killifish were exposed to 0, 50, 200, and 800ppb arsenic as embryos, and after hatching, were reared in clean water for up to 78 weeks. Growth, assessed by condition factor, was significantly reduced in the 200 & 800ppb groups by 17-29%. The reduction in growth was correlated to changes in IGF1 and IGF1R transcript levels. Behavioral changes were also noticed during the grow-out period, including reduced response to food and huddling at the bottom. So, two different odorants were used to assess responses. No changes were seen with taurocholic acid, a bile salt that elicits a social response. However, introduction of an amino acid mixture, which initiates a feeding response, significantly reduced both the duration of response by 50%, and percent of fish responding by 42%. We hypothesize that embryonic exposure to arsenic impairs the fish's ability to sense odorants, leading to a reduction in weight, which is potentially caused by changes in IGF1 signaling pathways and/or reduced uptake of nutrients. Discoveries from this study can help raise awareness of the implications arsenic exposure can have on a growing fetus and potentially lead to more stringent regulations on the amount of arsenic allowed in food and water sources.

TP116 Evaluating the fate and effects of selenium in lab-cultured and field-collected biofilms

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A weight of evidence approach has been employed to better understand the biological, chemical and physical influences affecting selenium (Se) fate and effects in biofilms. Biofilms were cultured in-house over a period of 4 months under continual exposure to Se and compared to field-collected biofilms from an area known to have elevated Se in sediments. The techniques used included mini-peepers, Diffusive Gradients in Thin films (DGTs), mineralogical characterization, sequential extractions and microbial assessments to understand the various influences affecting Se fate. In addition, a number of exposure assessments were conducted with surface sediments (field) and biofilms (lab) to evaluate the trophic-transfer of Se to the benthic invertebrate Chironomus dilutus. The results from this study provide a better understanding behind the bioaccumulation of Se in biofilms, its potential effects on biofilm communities and the drivers behind Se mobilization.

TP117 Expression of metallothionein cDNA as a biomarker of environmental pollution in the wild mussel Mytella guyanensis in the Gulf of Guayaquil, Ecuador

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The potential use of a biomarker (metallothionein) in the digestive gland of wild mussels (Mytella sp.) for ecotoxicological assessment and biomonitoring of environmental pollution in the Guayaquil Gulf Estuary (Ecuador) was investigated. The synthesis of metallothionein is a specific response to elevated concentrations of inorganic pollutants. Metallothionein is a cytosolic protein found in a variety of tissues, playing an important role in the detoxification of metals and as a biomarker of exposure to heavy metal contamination. The objective of this study is to observe the methallothionein expression in this wild mussels species exposed to contaminated mercury sites. Two locations from Gulf of Guayaquil: Reserva de Producción Faunística Manglares El Salado (RPFMS) and Reserva Ecológica Manglares Churute (REMCH) were selected. While the RPFMS is extensively affected by anthropogenic pollution (urban-industrial development, agricultural monoculture and more recently shrimp pond aquaculture) with an average sediment total mercury concentration of 1.027 mg/kg dw. The REMCH is considered as a reference semi-pristine area exhibiting an average sediment total mercury concentration of 0.05 mg/kg dw. The average concentration in mussels collected at the RPFMS was 3.480 mg/kgdw, which exceeds the wet threshold (0.5 mg/kg) safety value used by many countries. The relative expression of metallothionein gene in mussels exposed to contaminated sites was detected for 100 copies of the housekeeping gene (Mitella sp.-β-actina) by qPCR. The results of this preliminary study suggest that toxicological gene expression of metallothionein may be a candidate biomarker and a tool for further biomonitoring mussel exposure to environmental inorganic pollutants in this tropical region.

TP118 Freshwater bivalve shells reveal the behavior of trace element concentrations in space and time

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Trace elements pose a challenge to our ability to understand their distribution in that they occur naturally. Trace elements may accumulate in a system due to geological and chemical factors other than pollution, or through indirect effects caused by human activities such as land use changes. In order to fully understand these effects, data sets across a large temporal and spatial range are required, however most modern pollution monitoring programs were only instituted in the 1970s or later. In this study, mussel shells from prehistoric, historic and modern collections were analyzed as proxies of trace metal accumulation in the Illinois River system. Shells were found to have almost no variation between species collected at the same site. Prehistoric shells consistently accumulated higher concentrations of Mn and Zn, but lower concentrations of As, Co, Cu and Ni. The elements Cd, Co, Cu and Ni were found to have increased over the past 120 years, while Mn decreased. One potentially confounding factor was that older populations of shells were significantly older and larger than larger collections. However, allometric relationships were not consistently significant and had little effect on temporal trends. In contrast, allometric relationships had a large effect on analysis of the spatial gradient along the Illinois River mainstem due to strong allometric effects at the uppermost and lowermost sites. Overall, modern shells indicated higher downstream concentrations of As, Co, Mn and Ni. Elements that varied spatially in no direction was Co, Cu, Ni and Zn. Large scales in the watershed and bivalve populations have clearly occurred over time. These changes are clearly expressed in the concentrations of elements such as Mn, which accumulates in higher concentrations in older shells. Other elements show clear elevation over a prehistoric baseline and vary in time and space, such as As, Co, Cu and Ni. Finally, another set of elements appears to be extremely conservative with little temporal or spatial differences. This set includes Fe and Cd, which while commonly associated with pollution appears in shells at extremely consistent concentrations. This study can serve as a model for future investigations into trace metal concentrations in bivalve shells and works towards a greater understanding of the manner in which they vary in both space and time.

TP119 Hepatic metal-binding complexes in cytosolic and mitochondrial fractions of field-collected eels (Anguilla rostrata) as determined by SEC-ICPMS

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American eel (Anguilla rostrata) populations inhabiting the Saint-Lawrence River (Canada) have severely declined over the last 30 years. Metal contamination, among other factors, may have contributed to this downward trend. In this context, measurements of subcellular metal-partitioning have revealed that although this species can detoxify metals (e.g., binding of metals to thermostable cytosolic proteins, metal incorporation into granule-like structures), indications of potential metal-induced toxicity were observed. Inappropriate binding of some metals to physiologically-sensitive target molecules (e.g., cytosolic enzymes) and organelles (e.g., mitochondria) can occur even in fish having low metal concentrations. To determine the molecular entities involved either in metal the detoxification response or targeted by these contaminants, we applied a hyphenated fractionation approach, based on size-exclusion chromatography coupled to inductively-coupled plasma mass spectrometry (SEC-ICPMS), to the cytosolic and mitochondrial fractions, where high metal concentrations were reported in livers of A. rostrata. In the whole liver cytosol, cadmium-containing complexes were found in the medium molecular-weight pool (MMW: 33 kDa – “1.3” kDa), where metallothionein-like proteins or peptides (MTLPs) are expected to appear. In contrast, thallium was consistently associated with low molecular ligands (LMW: < “1.3” kDa). The mitochondrial fraction was lysed with Triton 1% and centrifuged to remove the mitochondrial membranes. In the mitochondrial matrix, cadmium was also bound mainly to ligands of MMW (~9.5 kDa), suggesting the occurrence of MTLP ligands within the mitochondria. In contrast, Tl in the mitochondrial matrix was bound to ligands of both high molecular weight (HMW: > 620 kDa – 33 kDa) and LMW. These results provide important insights into the intracellular trafficking of metals in aquatic organisms chronically exposed to non-essential metals.

TP120 How long does it take for selenium to bioaccumulate in the diet and tissues of sturgeon?

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In trying to answer the question “how much is too much” with respect to selenium in the San Francisco Bay-Delta, attention rightly has been focused on the introduced Asian overbite clam that bioaccumulates selenium especially efficiently, and on the vulnerable sturgeon species that feed on this clam. In the Bay-Delta environment of fluctuating selenium and migrating sturgeon, to assess the risks of selenium to sensitive life stages of sturgeon, it is essential to understand the time delay between exposure to selenium in ambient water, and bioaccumulation of selenium in the tissues of the sturgeon species. Hitherto, estimating these lag times has been a matter of guesswork. Now, a statistical method has been demonstrated that enables us to provide more objective estimates. The method was developed using selenium monitoring data from the Grassland Bypass Project in the Kesterson area of the San Joaquin valley. The method can be used to infer estimated selenium bioaccumulation lag times of roughly 50-120 days for overbite clams, 178 days for white sturgeon, and 247 days for green sturgeon. Reliability of these estimates will be discussed along with implications for risk of selenium to sturgeon species.

TP121 Identifying Metabolomic and Transcriptomic Impacts of Heavy Metals in Zebrafish (Danio rerio)

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Teleosts are valuable sentinel organisms for the aquatic environment as well as human health as they share a high degree of similarity in metabolic and physiological features with other vertebrates. As early life stages in fish have a reduced capacity to metabolize xenobiotics compared with that of mammals, embryonic or larval windows can be a sensitive toxicological model, particularly in addressing adverse effects of low-level chemical exposure. Compared with morphological assays, changes in mRNAs, proteins, and/or metabolite levels are sensitive and potential early indicators of systemic effects. As endogenous metabolite concentrations are determined by the organism’s biochemical processes, metabolomics is regarded as a comprehensive evaluation of biological response. Similarly, transcript levels can shed light on the transcriptional processes in the animal. In the present study, we evaluated the biological impacts of zebrafish larvae (Danio rerio) acutely exposed to environmentally relevant concentrations of heavy metals including, Cd, Cu, MeHg, and $AgNO_3$ using both metabolomics and transcriptomics. Quantitative measurements of 209 metabolites including amino acids, biogenic amines, glycerophospholipids, acylcarnitines, sphingolipids, fatty acids, bile acids, and ∑hexose from whole animals were performed by LC-/FI-MS/MS. Metabolomic profiles were distinguishable among chemicals. A strong concentration effect was observed in Cd and Cu exposed animals, where a general reduction in amino acids as well as docosahexaenoic acid was observed. In contrast, glycerophospholipids, sphingolipids, and ∑hexose, as well as GABA concentrations were significantly impacted in the $AgNO_3$ exposed animals but without a clear chemical concentration effect. Metabolite enrichment analysis in Cu and Cd exposed animals revealed affected pathways invovled in anti-oxidation protection such as selenoamino acid metabolism and glutathione synthesis, and in neuromodulation such as catecholamine biosynthesis. In addition, quantitative real-time PCR based transcriptomic analysis targeting 39 genes transcript presenting important developmental biomarkers will be performed. This combined method will provide the critical molecular level information needed to facilitate a systems biology approach in characterizing and evaluating potential biological impacts of heavy metal exposure in a teleost model.

TP122 Maternal transfer of metals in Steller sea lions (Eumetopias jubatus) from the Washington State coast

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Steller sea lion (Eumatopias jubatas) habitat extends throughout the perimeter of the Northern Pacific Ocean and is divided at 144ºW longitude. This species is distributed throughout Japan and Russia (the Eastern population) to the Aleutian Islands and California (the Western population). The population reduction in the Western stock and the population increase in the Eastern stock have been the basis of studies investigating intentional killing and entanglement, climate and dietary changes, disease, and contaminants as possible causes of this divergent pattern. Previous studies have evaluated metals concentrations in organ tissues that may cause neurological and other deleterious effects in Steller sea lion adults. Few studies have focused on the gestational transfer of metals between mothers and fetuses. Many metals are required for nutrition at low levels, and thus play a complex role in the metabolic processes of organisms. Due to maternal offloading, fetuses may endure more pronounced and problematic effects as a result of rapid biochemical integration of toxic contaminants during development. In the present study, metals concentrations were quantified in organs of four mother-fetus pairs located off of the Washington state coast to evaluate gestational transfer in the species. Samples were collected by the Washington Department of Fish and Wildlife – Marine Mammal Investigations Unit from deceased mammals by field researchers investigating other aspects of their life history. Comparisons of the mother's body mass and trace metal concentrations in her tissues will be evaluated. Further comparisons will be applied to her fetus. Metals of interest in this study include mercury, selenium, cadmium, and lead. To the best of our knowledge, this investigation will be the first to incorporate these metals in this context and will continue to expand on our knowledge of how metals are metabolized in Steller sea lions.

TP123 Metabolic and cardiovascular effects of dietary selenomethionine exposure in adult zebrafish (Danio rerio)

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Selenium (Se) is an essential micronutrient involved in important metabolic functions for all vertebrate species. As Se is reported to have a narrow margin between deficiency and toxicity, there is growing concern surrounding the adverse effects of elevated Se exposure caused by anthropogenic activities. Oviparous vertebrate species, especially fish, are highly susceptible to elevated dietary Se exposure. Recent studies have reported that elevated dietary exposure of fish to selenomethionine (Se-Met), the primary form of Se in the diet, can alter metabolic capacity, energy homeostasis, swimming performance and cause a greater incidence of early life stage deformities and mortality. This study aims to further investigate mechanisms of Se-Met toxicity, particularly potential underlying cardiovascular implications of chronic exposure to environmentally relevant concentrations of dietary Se-Met in adult zebrafish (Danio rerio). Adult zebrafish were fed either control food or Se-Met spiked food (10.27 or 28.81µg Se/g, dry weight) for 90 days at 5% body weight per day. Following exposure, high resolution B-mode and Doppler ultrasound was used to characterize cardiac and vascular function. Chronic dietary exposure to Se-Met caused significant decreases in blood velocity through the atrioventricular (AV) valve, reduced stroke volume and cardiac output, and impaired ventricular diastolic filling. These effects may lead to further cardiovascular complications. Metabolic endpoints investigated included muscle glycogen and triglyceride stores, and fish fed with Se-Met spiked food had elevated energy stores, suggesting impaired energy homeostasis and metabolic dysfunction. Expression of mRNA genes of interest was quantified by use of Real-Time Quantitative Polymerase Chain Reaction (qRT-PCR) and current findings are being examined. The results of this study suggest that chronic exposure to dietary Se-Met can alter both cellular and physiological responses, and such consequences could threaten fitness and survivability of fish.

TP124 Metal-induced olfactory sensory neuron death and regeneration in zebrafish

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Fish rely heavily on olfaction to maintain behaviors essential for survival, including predator detection and avoidance, prey selection, social behavior, imprinting, and homing. Environmental exposures to the metal ions copper (Cu) and cadmium (Cd) are known to cause olfactory toxicity with differential effects in olfactory neuron susceptibility and regeneration. In this study, zebrafish (Danio rerio) are used as a model system to study olfactory death and regeneration in teleosts. Confocal imaging in double-transgenic zebrafish larvae that differentially label ciliated and microvillous olfactory sensory neurons (OSNs) is used to image and analyze OSN changes following metal induced death and recovery. Following a 24-hour exposure to Cu at 5 days postfertilization, both ciliated and microvillous OSN populations exhibited cell injury and death in a dose-dependent manner. Recovery and regeneration of these OSN populations was observed at 24 and 48 hours after exposure. Cell proliferation assays using BrdU incorporation showed increased cell division following Cu-induced OSN death. Exposure to Cd also resulted in visible ORN death, albeit at higher concentrations relative to Cu exposures. Regeneration of OSNs was also observed 24 hours after Cd exposure. Assays for olfactory-driven behaviors were used to correlate the morphological changes observed through confocal imaging with behavioral endpoints. Our studies support the use of transgenic zebrafish to understand the mechanisms of olfactory injury and recovery that can occur in teleosts exposed to metals. Supported by NIEHS Superfund P42-04696.

TP125 Observed lower tissue residues of metals in Japanese dace collected from a metal contaminated river

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Heavy metal contamination is a major concern in freshwater environments. Accumulation (tissue residues) of metals in aquatic species has been well studied to evaluate the levels of the pollution and/or of the exposure of the studies organisms. The Watarase River, running in the northern Kanto region of Japan, had been severely polluted by heavy metals discharged from mining activities. Although the remarkable decrease in concentrations of metals including copper and zinc were observed, e.g., in 1960s, the concentrations notably in the upstream region are still higher than those in a reference river, the Omoi River. In the present study, toward understanding the biological responses of aquatic species to the current contamination, the metal accumulation (Cu, Zn, As, Pb, Cd, Fe) in muscle, liver, and gonad of Japanese dace, Tribolodon hakonensis (Cyprinidae) collected from two mid-stream sites in the Watarase and Omoi rivers, as well as those water and sediment concentrations, were investigated. Water and sediment concentrations at the site in the Watatase River were generally higher than those in the Omoi River. In contrast, interestingly, this trend was not necessarily observed for the metal accumulation in organs of Japanese dace. For instance, the amount of metals accumulated in liver of the dace in the Watarase River was often smaller. We will present detailed results on the metal accumulation and population genetic structure inferred from microsatellite genotyping, and discuss important implications for performing metal accumulation and toxicity studies.

TP126 Persistent lead contamination in an urban marsh: The legacy of lead shot

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The La Crosse River Marsh (LRM) is a unique urban riparian marsh recognized for its high biodiversity in an urban setting. The La Crosse Gun Club operated a large trap shooting range at the LRM between 1932-1963, resulting in significant quantities of lead (Pb) shot being discharged into the marsh. Pb-contamination remains high as we have found Pb shot densities of >43,000 pellets/m^2, and surface sediments contain as high as 23,000 ppb Pb in some areas of the marsh. Using a combination of field and laboratory studies, we used a multi-disciplinary approach to survey and assess the impacts these contaminated sediments have on the marsh ecosystem. Water in the marsh contained concentrations within the range of concern for chronic toxicity. Pb from contaminated sediments is bioavailable, and Pb levels within duckweed, invertebrates, and some fish species correlate with the Pb found within sediments. Our laboratory toxicity assays suggest that sediments are minimally toxic to developing fish following acute exposure. Since the lead is not mobilizing system wide, we suggest any remediation efforts be focused in specific areas of high concentrations of the LRM, and that continued monitoring of the marsh is warranted.

TP127 Polymetal mixture from mining pollution alters functional traits and metabolomic profiles of freshwater clams (Musculium spp.)

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A century of mining activity in the Coeur d'Alene, ID (CDA) region discharged an estimated 56 million metric tons of tailings into the CDA drainage. We are exploring whether freshwater clams (Musculium spp.) could serve as sentinels for the drainage. We collected clams (N=147) from an unimpacted site and exposed them (40 d) to water in equilibrium with lake sediment from the CDA drainage, with uncontaminated sand (control) or with a 50% sediment/sand mixture. Water in equilibrium with the 100% sediment treatment had 32 µg/L Cd, 411 µg/L Pb and 2,561 µg/L Zn. Functional traits were measured weekly and metabolomic samples collected at 24h, 48h, 72h, and weekly. Clams exposed to 100% sediment had the lowest survival ($p \leq 0.001$) and climbing ($p < 0.002$), maintained the largest brood sizes ($p = 0.002$) and extruded the fewest juveniles ($p < 0.05$). Of the functional traits, only juvenile extrusion followed a dose response pattern. We quantified 139 metabolites in a total of 35 samples from the control and 100% sediment treatments. Cystathionine and hydroxyproline tended to be lower in clams from the control conditions. Uridine, asparagine, aspartic acid, and 2-aminoadipate tended to be lower in sediment-exposed clams. These results indicate that metal exposure decreases clam activity, reproduction and survival and affects amino acid metabolism. A combination of functional traits and select metabolites may be effective biomarkers for metal exposure in these clams.

TP128 Responses of Calcifying Organisms to Ocean Acidification and Copper Exposure

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Ocean acidification and land-based sources of pollution have both been linked to widespread declines of coral cover in coastal reef ecosystems. At the current rate of change, atmospheric CO_2 levels are predicted to increase to 1000 ppm by the end of this century. Though much attention has been focused on ocean acidification and to some extent metal pollution, only limited research has addressed combined exposure of both pollutants to coral reef organisms. In this study, two coral species, Acropora cervicornis and Pocillopora damicornis, and the sea urchin, Strongylocentrotus droebachiensis were exposed to two copper concentrations at two CO_2 levels (ambient and 1000 ppm) for 96 h. Cu accumulation and activities of the enzymes catalase, glutathione peroxidase, glutathione reductase, and carbonic anhydrase were measured. The results provide insight into mechanisms of both Cu and CO_2 toxicity to coral reef organisms and have implications for environmental exposure of multiple contaminants.

TP129 The effect of hydropeaking on energy stores and mercury concentrations in shoreline dwelling spottail shiner (Notropis hudsonius)

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Hydroelectric reservoir construction induces chemical and physical changes that can affect downstream ecosystems. Methylmercury produced in reservoirs rapidly bioaccumulates in reservoir-impounded and downstream foodwebs, as evidenced by significant post-dam declines (1970 to 2013) in Hg concentrations ([Hg]) of commercial fishes (pike, walleye, sauger, and goldeye) from the Tobin Lake Reservoir and a downstream fishery. Further analyses reveal significantly delayed [Hg] decline from downstream walleye and goldeye populations relative to those in the reservoir ($p < 0.001$) despite low contemporary [Hg] in water. These latter effects could be related to stress imparted by daily dam-induced water fluctuations. Fish kills and strandings observed on low-pitched shorelines below the dam suggest that the irregular and highly variable shoreline manipulations imposed below this peaking hydro-electric facility may act as a chronic physical stressor, causing increased retention of [Hg] in downstream fish. Analyses of triglyceride and Hg concentrations in young of the year spottail shiner (Notropis hudsonius) collected from affected habitats reveal reduced energy accumulation in young of the year fishes ($p < 0.05$) and relatively greater [Hg] ($p < 0.05$) compared to upstream counterparts, suggesting that hydro-electric facilities may induce an energetic bottleneck that exacerbates [Hg] in downstream fishes. Analyses of glycogen depletion and cortisol induction in response to an acute physical stressor are currently underway to determine whether fish from affected habitats exhibit physiological signs of chronic stress.

TP130 The kinetics of selenium accumulation in laboratory algal cultures and implications for extrapolation to the field

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Selenium accumulation in algae varies with many factors, including selenium species, algal species, and aqueous selenium and sulphate concentrations. Large differences in accumulation have also been observed between laboratory cultures and natural algal assemblages in the field. We used a kinetic analysis to explore how these factors could explain observed variability in bioaccumulation datasets. We measured selenium accumulation in the green alga Pseudokirchneriella subcapitata at a range of selenium and sulphate concentrations and modelled the effect of water chemistry on the uptake rate constant. We also measured algal growth and calculated the contribution of growth dilution to total elimination rate. We combined these data with published data on selenium uptake kinetics in other algal species and with field data characterizing selenium accumulation in natural algal assemblages. Our analysis suggests that growth can explain differences in selenium accumulation between lab and field, and could account for much of the observed variability in field data. The effect of sulphate, mediated by selenium:sulphate ratios and selenium speciation, may account for regional and habitat-related differences in bioaccumulation.

TP131 Trophic transfer of three-layered silver nanoparticles (Ag@gold@Ag) from the diatom Nitzschia palea to the snail Lymnaea stagnalis

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For silver nanoparticle (Ag NP) exposures, the role of the ions and particles in Ag bioaccumulation and toxicity has not been fully elucidated, with many reported studies suggesting that only Ag ions are taken up. Furthermore, very little is known about the influence of ions and particles for dietary uptake. Here we use an innovative tool, i.e., a three layered core-shell-shell Ag NPs (Ag@Au@Ag) to address the particle versus ion uptake question in feeding experiments with a freshwater invertebrate grazer. The Ag core of the NPs was prevented from dissolution by a gold (Au) layer, while the outer Ag layer coated with polyvinylpyrrolidone (PVP) could dissolve. Specifically, we exposed the pond snail Lymnaea stagnalis to diatoms mixed with increasing concentrations of Ag@Au@Ag NPs and measured Ag and Au uptake rates in the snail's soft tissues. Mass balance calculations of the Ag and Au allowed inferring food ingestion rates and assimilation efficiencies, which were used to estimate bioavailability. Comparisons of Ag/Au ratios in water, diatoms, snail's soft tissues and feces allowed determining whether Ag@Au@Ag NPs, Ag ions and/or Au are absorbed by the snails. Results so far suggest that nanosilver exposure from food might trigger important risks. Three layered core-shell-shell Ag NPs are novel tools to help understand these risks.

TP132 Use of Benthic Macroinvertebrate Field Collected Data in the Derivation of Site-Specific Standards for Iron in Circumneutral Waters

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The field-based portion of this study was initiated in 2014. The purpose was to evaluate whether the existing concentrations of iron present in select, non-acid mine drainage stream segments in northwestern Colorado, even if they exceed the Table Value Standard of 1 mg Fe/L on the basis of total recoverable metal, are likely to pose a risk to aquatic organisms. Since 2014, on an annual basis, GEI has been collecting benthic macroinvertebrate samples from 17 – 25 sites concurrent with extensive water chemistry data in order to assess the health of aquatic communities and characterize the associated water chemistry profile. Additionally, a measurement of the potentially dissolved fraction and a sample collected using a 10 µm capsule filter has been analyzed to explore the use of alternative analytical methods. Sites selected consisted of locations characterized by high iron conditions matched with appropriate reference locations containing low iron, but similar habitat, flow, and total suspended solids concentrations. In order to properly partition the factors influencing the structure of the macroinvertebrate community, sites were targeted which worked to evaluate multiple variables including both disturbed and undisturbed locations within these watersheds, as well as segments with ephemeral and intermittent flow regimes. Based on data analyzed to-date we expect that a biologically relevant standard can be developed based on iron concentrations that are not associated with adverse impacts on benthic community structure when compared to nearby reference sites – potentially resulting in something akin to an ambient-based standard. Study objectives, design, and initial data evaluation will be presented.

TP133 Analysis of Se in pectoral fin rays of white sturgeon (Acipenser transmontanus) from San Francisco Bay as a non-lethal assessment technique

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There is increasing pressure to develop alternative methods to assess the hazards posed by contaminants to wildlife while also reducing the number of lethal sampling events using wild animals. Opportunistic sampling was conducted to collect tissues from angler-harvested resident white sturgeon in San Francisco Bay, with the ultimate goal of developing a non-lethal method to assess exposure to inorganic contaminants. Specifically, this population of sturgeon is thought to be at risk to accumulate elevated concentrations of Selenium (Se) from their diet, which predominantly consists of overbite clams (Potamocorbula amurensis). Soft tissues (eg., muscle or egg/ovaries) are typically analyzed to determine Se exposure, but ongoing excretion, metabolism and tissue redistribution preclude resolving any temporal information from these analyses. Time resolved exposure can be determined using micro-chemical analysis of otoliths, but this requires lethal sampling. Pectoral fin rays also contain annual growth rings, but can be sampled non-lethally and require no special preservation for micro-chemical analysis. However, the relationship between trace elements in the bony matrix of otoliths and fin rays is not yet known. As part of a collaborative study, 27 and 22 adult white sturgeon were collected from San Francisco Bay in Feb 2015 and 2016, respectively. Selenium, as well as Zn, Sr, Hg, Pb and Ca (internal standard) were analyzed in annual growth zones of pectoral fin rays and otoliths from these fish using continuous LA-ICP-MS. Relationships between concentrations of Se in soft tissues (muscle and ovary), otoliths and pectoral fin rays will be presented. Fin rays may represent a relatively simple non-lethal tissue sample to establish temporal exposure to several elements in white sturgeon from San Francisco Bay.

TP134 Assessment of heavy metals concentration in fish tissues of selected fish species in Oguta Lake, Southeastern Nigeria

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The study was carried out in Oguta Lake from January, 2012 to December, 2013 at five stations (Onu Utu, Okposha, Ogbe Hausa, Osemotor and Ede Ngwugwu) to study the concentration of heavy metals in fish tissues. The Lake is the largest natural freshwater lake in Imo State, Nigeria and originated from a natural depression. It is bounded between longitude 6° 41' – 6°50' East and latitude 5°41' – 5°44' North of the equator. Fish samples were collected twice per month using hook and line, gill net, cast net, bag net and local traps. A total of 1,989 fishes were used. The levels of heavy metals Copper (Cu), Lead (Pb), Cadmium (Cd), Arsenic (As) in three common edible fish species (Tilapia zillii, Citharinus citharus and Heterotis niloticus) were determined by air-acetylene flame Atomic Absorption Spectrophotometer after wet digestion of samples with 1:1 HNO_2, H_2O_2. The mean values of the heavy metals from the study shows; 0.67µg, 0.58µg, 0.64µg and 0.21µg for Cu, Pb, Cd, As respectively. The results obtained showed that the values of all the heavy metals in the fish samples were lower than the values recommended by WHO and the Food and Agricultural Organization of the United Nations and therefore safe for human consumption.

TP135 Bioaccumulation and trophic transfer dynamics of heavy metals in food web assemblage of intertidal mangrove ecosystem, Sungai Puloh, Malaysia

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In recent times, the ever increasing anthropogenic activities associated with industralization, population increase and urbanization in developing countries which leave chemical foot prints behind, pose risk to aquatic ecology. Among this chemical pollutants are heavymetals which are bioaccumulative, persistent, and toxic. this consequently impact aquatic organisms through bioaccumulation, trophic interactions, and dietary exposure. The aim of this research is to evaluate the heavy metals (Cu, Pb, Ni and Zn) and to assess their bioaccumulation patterns in surface sediment and some key ecological food chains in Sungai Puloh estuary. Also to characterize the trophic positions of samples: Periophthalmodon schloserri,Osteogeniosus militaris, Telescopium telescopium, Nerita lineata, Polymesoda coaxans,Penaus monodon,Uca annulipes, Dotilla myctiroides,and planktons in food web structure of Sungai Puloh. The direct aqua regia method was employed for metal estimation and metal concntrations (micro gram/g, dry weight)were determined by using an Air-acetelyne flame Atomic absorption flame Spectrophotometer, by Perkin- Elmer, and Isotope Ratio Mass spectrophotometer was used for stable isotopes ratio determination. The result revealed elevated Cu in P. mondon (47.19) and high levels of Pb (15.23, 16.86, 8.91) in catfish (O. militaris), U. annulipes, and D. myctiroides respectively. With the help of stable isotoe ratios of ^{13}C and ^{15}N analysis, two distinct food chains and four trophic positions were determined, with catfish occupying the highest trohic level. Using the catfish as a model for food web trophic system, the result showed that the carbon source for catfish (-17.30 per mill) is from the crabs (-17.39, -18.03) per mill for Ucan and Dotilla respectively with an enrichment of ^{13}C ratio values ~I per mill, complementing the idea that crab found in the stomach content is its staple food. It is arguable that the major source of Pb in catfish is from crabs and not from surface sediment since the biota sediment accumulation factor (BSAF: 0.2) recorded very low ration compared to that of the trophic tranfer factor (TTF:1.7). We concluded that heavy metal contamination in Sg. Puloh stems from the release of untreated or poorly / improperly treated effuluents into this mangrove ecosystem, and these metal contaminats are evidenced in the food chains of Sg. Puloh mangrove. The Pb in this study area pose some serious risk, and should be srictly monitored.

TP136 Determining Mercury Concentrations and Evaluating Two Biomarkers of Exposure in Two Popular Recreational Fishes in Hawaiian Waters

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Mercury concentrations have been measured in Hawaiian commercial pelagic fishes and some bottomfish species, but have not been measured in many nearshore fish species targeted for recreation and food. Monitoring the commercial and local food fishes around the Hawaiian Islands is necessary to identify possible threats to the ocean environment and human health. Depending on the concentration and the chemical form, mercury exposure in fish can have lethal and sub-lethal effects including changes in behavior, reproduction, feeding, and tissue pathology. When assessing the risks of mercury exposure on aquatic ecosystems, molecular biomarkers are used to examine adverse effects. Metallothionein mRNA expression and protein levels increase in fish tissues through waterborne and dietary mercury exposure. Thioredoxin reductase mRNA expression levels have been shown to increase while protein activity was inhibited in fish tissues from waterborne mercury exposure. The purpose of this study was to measure mercury concentrations in two groups of nearshore fishes, trevallies, Caranx sp., and bonefishes, Albula virgata and Albula glossodonta, in order to provide baseline data for evaluating mercury exposure in these popular recreation fish. Trevally muscle and liver mercury concentrations ranged from 51.8 to 610.7 and 37.9 to 1501.2 ng/g wet mass, respectively. Bonefish muscle, liver and kidney mercury concentrations ranged from 93.3 to 937.8, 55.9 to 2390.4, and 92.4 to 5220.0 ng/g wet mass, respectively. In addition, thioredoxin reductase and metallothionein levels were measured as potential biomarkers of mercury exposure and initial assessment of fish physiological responses to mercury. Quantitative real-time PCR was used to measure mRNA expression levels and western blotting was used to measure protein levels to evaluate the potential use of these physiological responses as biomarkers of mercury exposure.

TP137 Embryonic-only arsenic exposure reduces growth and alters skeletal muscle development in killifish (Fundulus heteroclitus) one year later

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Arsenic is a contaminant of drinking water and crops in many parts of the world. Epidemiological studies have shown that arsenic has been linked to decreased birth weight, weight gain, and proper neuronal and skeletal muscle function. The goal of this study was to determine the effects of embryonic-only arsenic exposure on muscle growth and behavior in killifish up to one year after the exposure ends. Killifish embryos were exposed to 0, 50, 200 or 800ppb As^{III} from fertilization until hatching. Juvenile fish were reared in clean water and muscle samples were collected at 16, 28, 40 and 52 weeks of age. Feeding behavior was assessed at 28, 40 and 52 weeks of age. There were significant reductions in condition factors (CF) in the fish exposed to arsenic at 16 weeks (12-17%) and 28 weeks (12-15%). By 40 weeks, when the fish were able to be sexed, male fish had 13-15% reductions in CF at 40 weeks, while females had a 10-12% reduction at 52 weeks. Feeding behavior analysis showed quicker occupation of the top of tank in controls compared to arsenic-exposed fish, and consistently higher occupation of the bottom of the tank in the 200 and 800ppb fish. IGF-1 levels significantly correlate with condition factor in control fish, but this relationship dissipates as arsenic concentrations increased. Based on these results, arsenic is impairing growth and altering skeletal muscle development in killifish up to one year after an embryonic-only exposure. This could have implications in regards to arsenic exposure through maternal drinking water and fetal development.

TP138 Evaluation of the Effect of Cadmium (0.2mg/l) on Catfish (Claria garaiepinus)

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This study was designed to examine the effect of cadmium and Hibiscus sabdariffa calyx extract on cat fish (Clarias gariepinus). In this research work, four experimental set up were carried out and labeled group I, group ii, group iii, and group iv. Group I contains 30 liters of water and fish only (control group),group ii contains 30 liters of water, fish and cadmium only, group iii contains 30 liters of water, fish, cadmium and the extract, while group iv contains 30 liters of water, fish and extract only. From this study, exposure of 0.2mgl of $CdCl_2$ did not cause significant ($P > 0.05$) increase in the gill-body weight ratio relative to the cadmium free (control). Administration of $CdCl_2$ plus Hibiscus sabdariffa extract caused significant ($P< 0.05$) increase in the gill-cholesterol concentration and also the extract only caused insignificant ($P > 0.05$) increase in the gill concentration relative to the $CdCl_2$ plus Hibiscus sabdariffa extract free group. On administering both $CdCl_2$ and Hibiscus sabdariffa extract, they were found to produce gill-body weight ratio and gill-cholesterol concentration that are significantly ($P < 0.05$) different from the $CdCl_2$ plus Hibiscus sabdariffa extract free group. In this study, it was observed that Hibiscus sabdariffa calyx extract lowers cadmium induced high gill cholesterol. Also established, is the fact that gill cholesterol status can be used as index of cadmium-induced physiological stress in the catfish.

TP139 Mercury bioaccumulation in mangrove Mytella sp. as a bio-indicator of environmental pollution in the Guayaquil Gulf Estuary, Ecuador

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The Gulf of Guayaquil is the largest estuary along the Pacific coast of South America with a surface area of approximately 13,701 km^2 and coastal line of 230 km. The Gulf contains approximately 81% of the total area of Ecuadorian mangroves, encompassing about 150,000 hectares, and a highly productive ecoregion for artisanal and industrial fisheries. Two protected areas of the Gulf of Guayaquil, the Reserva de Producción Faunística Manglares El Salado (RPFMS) and Reserva Ecológica Manglares Churute (REMCH), were subject of this study. Both reserves differed in the degree of land use and urban development. The RPFMS is affected by anthropogenic impacts showing an average total mercury sediment concentration of 1.027 mg/kg dw, exceeding the Effects Range Medium (ERM) sediment quality guidelines for Hg (0.71mg/kg). The REMCH is considered as a control area with an average total mercury sediment concentration of 0.05 mg/kg dw. The aim of this study was to assess mercury bioaccumulation in the mangrove mussel Mytella sp. using the biota sediment accumulation factor (BSAF = the ratio between the mercury concentration in the mussels and the mercury concentration in the sediment) in both reserves as a monitoring approach for environmental risk assessment. Preliminary findings revealed an average mercury concentration of 4.896±0.76 mg/kg dw in mussels collected at the RPFMS, exceeding the wet threshold (0.5 mg/kg) safety value set by several countries. The BSAF for this specie was 5.34 ± 2.62 (kg/kg). Characterization of chemical pollutants and ecological risk assessments of anthropogenic activities in Ecuador are needed to establish proper environmental management strategies to mitigate and control anthropogenic pollution. Future directions are also required to understand the fate, transport and exposure in the environment of these contaminants.

Considering Water Scarcity for Environmental Decisions

TP140 USEPA's Life Cycle Assessment Research Center

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Recognizing Life Cycle Assessment (LCA) as one of the key tools which quantifies potential environmental impacts to support sustainable decision making within the Program and Regional Offices, the USEPA has developed the LCA Research Center (RC) to strategically coordinate ORD's LCA-related staff, fiscal assets, communication, and research projects. Building upon the existing and growing LCA and sustainability expertise, the LCA RC conducts research to advance LCA methodologies, tools, data, case studies, and training that can be used by internal and external stakeholders. The current research program will be presented along with technical points of contact for each project. The multi-million dollar transdisciplinary program includes data management, web-based tools and impact assessment research. Our current research portfolio includes: near-field exposure modeling integrated with LCA, water scarcity, nutrient loading, water treatment alternatives, sustainable materials management, and decision support research and tool development. In addition to this research, LCA RC members are currently active in SETAC NA LCA Advisory Groups (chair), the SETAC Global Advisory Group on Sustainability (co-chair), the SETAC LCA Global Coordinating Group, the UNEP-SETAC Life Cycle Initiative International Life Cycle Board, the Federal LCA Commons, and as advisors within various LCA standards. The core members of the LCA RC include experienced and developing LCA and sustainability experts who have developed workshops, special sessions, and presented numerous presentations, in addition to publishing over 20 peer-reviewed research manuscripts in the past 18 months.

TP141 What if Water Footprints were a Social Construct? Should they still be done?

C. Stahl, USEPA Region III; A. Henderson, USEPA / ORD

No single tool will be good for all situations but when practitioners understand first principles, it becomes possible to evaluate any tool against the need and determine whether there is a match. The principles for constructing a water footprint or any other type of index, whether for Life Cycle Analysis or something else, are the same. The people who make the choices about what parameters are included (or excluded) in the index determine the context, which is the key. In this presentation, learn how understanding the principles of index construction allows users (one kind of stakeholder) to examine and challenge their implicit assumptions about indices and their own index. Starting with a simple example of the EPA Hazard Ranking Score, learn why this calculation is not purely scientific. Utilizing critical thinking, learn why it is necessary to embrace our humanness but to do it appropriately. Learn also that when we are inclusive, transparent, learning-based, deliberate and methodical, it is possible to embrace our human qualities without giving in to arbitrariness or capriciousness. Building indices (or footprints) are social (i.e., values-laden) constructs and not scientific ones, even as scientific information is used. Therefore, it is not the tool or the index that determines whether decisions using them will be robust but the people who understand how to think critically and analytically about how they are used. Committing to a new way of thinking could be more difficult than committing to building new tools but it might be the real solution. Further, once the principles for index construction are understood, it is then possible to similarly apply them to environmental decision making. Come to this presentation and learn why.

TP142 Considering water quality in the impact assessment of water use in industry

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Taiwan is an island country and depends on the electronics industry to create the booming economy. However, this industry not only requires large amounts of water, but also emits pollutants to water. And these activities deprive downstream water users, especially the agricultural sector, of enough and functional water. Even though the environmental impact assessment (EIA) has been implemented here, the trade-off between environment and economy is still not easy to be solved. The information provided by modeling the quantity of required water and the quality of emitted water is insufficient to support decision making. To identify the influence of industrial developments, an impact assessment model is needed. Therefore, based on life cycle assessment (LCA) framework and the withdrawal to availability (WTA) concept, this study developed a model which estimates the quantity and quality of water separately to represent water scarcity. And the model was applied to the case study which included several operating Science Parks in Taiwan. The result shows that the quality of water which cannot meet the requirement of downstream users would also result in water scarcity. Since high water scarcity relating to water quality does not stop water withdrawal by downstream sectors, hazards may rise by using low quality water. Thus, this model can clearly display the impact route of industrial activities and provide more information for further water resource management.

TP143 Characterisation model for green water scarcity footprint

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Terrestrial ecosystems mostly rely on green water which itself depends on precipitation, soil physics, climate and land use. Green water refers to precipitation on land that does not runoff or recharges the groundwater but is stored in the soil or temporarily stays on the top of the soil or vegetation. The consumption of green water in land use activities, per se, does not necessarily lead to a reduction in surface water and groundwater or contributes to water scarcity. Indeed, the potential impacts on water availability depend clearly on local land-use production systems, and on the natural vegetation that is replaced by land-use production systems. This is an issue that has been poorly studied in life cycle assessment because the environmental mechanisms of green water consumption are not well understood. These mechanisms are: (1) potential disturbances of regional long-term availability of surface blue water (called as green water and soil interface) due to changes in green water use, and; (2) potential perturbations in the evaporation and transpiration that is recycled into the atmosphere, and then, in the rainfall that returns to the regional terrestrial ecosystem (called as green water and atmosphere interface), due to land use changes. This study is devoted to the development of indicators of green water scarcity at 30-arcmin global grid, considering these interfaces to evaluate the environmental impacts of net change in green water use, in life cycle impact assessment (LCA). The developed green water scarcity indicators are based on the quantification of the net change in evapotranspiration of the land-use production systems compared to the potential natural vegetation (reference situation), taking into account the share of the water evaporated or transpired through plants to the atmosphere that returns to the river basin, and the global annual environmental flow requirements (EFR). EFR quantify the water flow required to sustain positive functioning of the blue-water-dependent aquatic ecosystems services, including those required for human livelihoods and well-being. By this way, we evaluate how green water demand and availability can change blue water production. The presented LCIA method is useful for environmental decision-support in the production of rain feed agriculture and forestry, allowing to accounting for water scarcity in decision-maker's environmental calculations in a consistent and acceptable manner.

Water Management: Focusing on Issues Beyond Simply Capacity

TP144 Holistic regional management of multiple water quantity, quality and other stressors in Africa using PROBFLO

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Best practice principles of Integrated Water Resource Management (IWRM) in Africa includes the need for robust holistic frameworks that address the risk associated with multiple stressors to social and ecological objectives on regional, trans-boundary scales. Recent rapid increases in water resource developments throughout Africa has resulted in widespread ecological impacts with associated socio-economic consequences. With regional regulators we have developed a regional IWRM Environmental flows framework that gives adequate consideration to non-flow stressors to describe the risk associated with sources in a holistic context. This regional scale ecological risk assessment approach incorporates Bayesian Networks to model the probable relationships between the flows and other driver variables, and socio-ecological indicators by assigning magnitudes and probabilities of adverse impacts of hazards to endpoints. In this paper we demonstrate the application of the PROBFLO approach in the Lesotho Highlands Water Project (Phase II) which includes the construction of the large Polihali Dam on the Senqu River, Lesotho and South Africa, and highlights from the Mara River application of PROBFLO as a part of the Water Allocation Plan for the Mara River Basin, Kenya and Tanzania. These case studies includes the establishment of Resource Quality Objectives for both case studies, the use of existing evidence, specialist solicitations and data generated through biophysical assessments to the study area. Outcomes included the establishment of a transparent, adaptable socio-ecological model to represent the risk relationships between sources, stressors, receptors and endpoints. A series of alterative management options and associated trade-offs were evaluated in the form of a range of scenarios with associated socio-ecological consequences. Scenarios were presented to stakeholders representing society who with the risk assessment outcomes contributed to the establishment of management plans for the use of the water resources. A range of mitigation measures were identified to minimise ecological consequences and monitoring. And adaptive management measures were designed to contribute to the implementation of the management plans. The PROBFLO approach has successfully allowed water resource use regulators to develop holistic plans to manage water quality, quality, habitat and other stressors on regional scales in Africa.

Advancing Sustainability in SETAC: Implementing the Berlin Declaration

TP146 A Rural Community's Pursuit of Sustainability

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I live in rural southern Alberta, Canada next to a town of 2200 people, 3 km from another town of 2400 people, with surrounding isolated enclaves of ranchers, labourers, and artisans. The lures of the area are many including a vibrant community of artists and artisans; stunning vistas of the foothills vegetation and Canadian Rockies; an abundance of wildlife; wild fisheries, and more. Over the last 100 years, the region was shaped by ranching, logging, and development of the first major oil and gas formation in western Canada. Farming and gardening are prominent in the area and there is an active contingent of permaculture practitioners re-envisioning the ways crops are grown. This area, referred to as Diamond Valley, is described by some as being in the "blast zone" of Calgary, a multi-cultural city of 1.3 million that has grown explosively in the past two decades and looks to be continuing to extend into the countryside. Residents of the Diamond Valley have varied interests, including ordinary business types and elected officials who seek economic growth. But there are others who desiring the serenity that currently exists advocate consideration of no-growth options. Over the past few years, visioning meetings have been organized to attempt to define desired futures for the area. Lively discussions about these futures have emphasized the importance of personal and collective community wellbeing. From these, a number of initiatives have been proposed; some already being acted on. These include establishing shared walking and bike paths between communities, promoting smart water management/conservation, passing municipal bylaws allowing the raising of "urban hens" for personal egg production, and an energy co-op that will use proceeds to promote energy efficiency, renewable energy, and other initiatives designed to foster community engagement and community wellbeing. In this presentations, the local effort will be used to illustrate the importance of stakeholder engagement in shaping sustainability goals and activities. Comparisons will be made to comparable efforts in other communities that are making positive gains in the areas of sustainability and community wellbeing.

TP147 A US analysis of dairy, agriculture, and overall nutrient cycling to identify improvements in nutrient use efficiency

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Article 2 of the SETAC Berlin Declaration calls for promotion of resource-efficient economies. Nutrient efficiency is an area of concern for agriculture, and indeed larger economic systems. Analyses of global nitrogen flows have identified the potential for increased efficiency from livestock; dairy, specifically has an important role in nutrient cycling and delivery of nutrients to consumers. This project aims to create national-level nitrogen (N) and phosphorus (P) budgets for the US, assessing the role of dairy and other livestock production in national nutrient flows. Results show that the combined fertilizer and chemical industry is the major fixer of N2 from air, with an annual fixation of 9,350 kt N/ yr. Agriculture is the second largest sector, with an N2 fixation of 5,156 kt N/ yr for US. Agriculture is the largest source of reactive nitrogen emission to air, predominantly due to ammonia emissions of 3,047 kt N from fertilizer application to field crops. Many feedback loops exist in both the dairy food supply chain and the 'non-dairy' food supply chain. For example, 104 kt N is produced as a by-product at the crop-based food industry and fed to the national dairy herd. N-efficiencies range from 71% for the poultry processing industry to 95% for the dairy processing industry. In total 1,203 kt N in synthetic fertilizer is used to produce 445 kt N in milk and 1 kt N in meat (boneless equivalent, veal calves only). Thus, 37% of total N applied with synthetic fertilizer ends up in milk supplied to the processing industry. In terms of nutrient use efficiency (NUE), the dairy processing industry is the most efficient part of the dairy food supply chain with an efficiency of 97%. Retail has an NUE of 92%. The least efficient part of the dairy supply chain is the dairy herd, with only 21% of nitrogen fed to the herd recovered in milk and meat. The crop production stage and household stage have intermediate NUE of 78%. 'Hotspots' in the dairy food supply chain occur predominantly in the crop production stage and the 'dairy herd' (milk production) stage, suggesting that improvement should focus in particular, on reducing ammonia emissions in manure management systems and at fertilizer and manure application on the field.

Recent Advances and Trends in Poly- and Perfluoroalkyl Substances Research

TP148 Monitoring of long-range transport of perfluoroalkyl acids in precipitation from the Newfoundland and Labrador Boreal Ecosystem Latitudinal Transect

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Perfluoroalkyl acids (PFAAs) are recalcitrant compounds globally disseminated among abiotic and biotic media (1,2). Gas phase PFAAs can form in the atmosphere by oxidation of volatile precursors, such as fluorotelomer alcohols (3). PFAAs are volatile in the neutral form, but due to low pK_a values (4), they should be ionized under most environmental conditions. PFAAs have low volatility in the anionic state, so it is likely the condensed phase, including atmospheric particles, plays a role in long-range transport. Rain droplets are effective scavengers of particulate-bound and gaseous PFAAs in the atmosphere. The measurement of precipitation in remote locations can provide a PFAA atmospheric profile, which will enhance interpretations of long-range transport. In this work, precipitation was collected over two years along four Newfoundland and Labrador Boreal Ecosystem Latitudinal Transect (NL-BELT) sites ranging from rural to remote areas. The sites are spaced along 5 degrees of latitude, extending into the sub-Arctic. Samples were collected using two precipitation sampler designs. The first design employs a large diameter tube for passive collection. This sampler is continuously exposed to the environment over the entire year, enabling the collection of wet and dry PFAA deposition. The second design employs custom-built automated precipitation samplers, which selectively collect wet PFAA deposition as incident and canopy-throughfall measurements. Differences in PFAA deposition profiles using these sampling designs could provide insight into dry deposition inputs into the NL-BELT. In order to accurately quantify PFAAs in precipitation, liquid chromatography-tandem mass spectrometry was used as the method of detection. Spatial, temporal, and homologue trends will be discussed, as well as the implications for mechanisms of long-range transport. The measurement of PFAAs in precipitation from multiple sites using different collection methods will help to understand long-range transport processes.

TP149 Analysis and screening of per/polyfluoroalkyl substances in a coastal urban ecosystem in California

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Per/polyfluoroalkyl substances (PFAS) have uniquely desirable properties that ensure their usage in many commercial applications. However, these properties also cause PFASs to be environmentally persistent, bioaccumulative in wildlife and humans and recalcitrant during water and wastewater treatment. The presence of several PFASs has been shown in wastewater and drinking water while manufacturers continue to introduce newer alternatives to the market. This study aimed to characterize the PFAS load into an urban coastal ecosystem through water, fish and sediment monitoring. A targeted approach using an LC-MS/MS was utilized to measure 11 PFAS including perfluorocarboxylic acids (PFCAs), perfluorosulfonic acids (PFSAs) and fluorotelomer acids (FTCAs) in each of the three matrixes at several points across the urban waterway. Concentrations in the water were low ng/L but PFOA and PFOS were detected while the sediment and fish had ng/g levels of total PFAS. While PFOA and PFOS were the most predominantly detected compounds, longer chain PFAS were also detected especially in the sediment. The fish sampled had higher levels of targeted PFAS and were further investigated using an untargeted approach with a UHPLC-QTOF/MS to identify other perfluorinated compounds while also screening for >1,000 organic contaminants including pharmaceuticals, hormones, pesticides and others using a database and library. The results of this screen provide a qualitative representation of the organic flux in the urban coastal ecosystem. Further, the identification of other potential perfluorinated compounds that may have been ingested in the fish were proposed and statistical analysis of the fish samples was performed to identify other organic contaminants while differentiating fish collected at different points across the urban waterway. The results of this study provide an idea of the perfluorinated compound loading and overall organic contaminant flux in an urban coastal ecosystem that will inform future monitoring programs.

TP150 Occurrence and composition of Per- and poly-fluoroalkylated substances (PFASs) in wastewater for direct potable reuse

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Faced with freshwater shortages, wastewater is increasingly utilized for the augmentation of water-starved environments. With the emergence of wastewater effluent as a viable alternative source for potable water use, additional research is needed to further characterize the contaminants of emerging concern (CECs) present in wastewater effluent and the risks associated with exposure to these contaminants. EPA Office of Research and Development (ORD) and EPA Region 6 publicly Owned Treatment Works (POTW) have partnered on a research project focused on waterborne pathogens and CECS that are present in wastewater effluent from advanced wastewater treatment plants. This project will assist the States in Region 6 that use wastewater effluent as a drinking water source by providing data on the occurrence and composition of potential waterborne pathogens and CECs. In this project, we will determine the types of pathogens and CECs that are found in wastewater effluent and the potential impacts (e.g. increase in levels of contaminants) of using wastewater effluent as a drinking water source on the overall water quality of the drinking water produced. This presentation will focus on the occurrence and composition of per- and poly-fluoroalkylated substances (PFASs) in the wastewater used in direct potable reuse. Monthly samples are collected and characterized for PFASs. The seasonal concentration and composition of these PFASs from wastewater influent and effluent of three POTWs will be reported to date. The PFASs concentration vary among plants. Summing the ten PFASs, the total concentration of PFASs in POTW1 were higher in both wastewater influent and effluent compared to other plants. PFOS, PFOA and PFHxA are commonly detected analytes with higher concentrations in wastewater influent. Other PFASs showed increasing concentrations during treatment processes with higher concentrations in wastewater effluent compared to the influent.

TP151 Analysis of poly- and perfluoroalkyl substances (PFASs) in drinking water: compliance with new USEPA Health Advisory Guideline

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Our recent study showed that serum concentrations of PFOS and PFOA in California women were linked to their presence in their drinking water supplies (as reported by UCMR3 (The third Unregulated Contaminant Monitoring Rule)). This finding, coupled with the May 2016 USEPA Lifetime Health Advisory Level for PFOA and PFOS (individual or combined) of 70 ng/L, warrants developing a method for drinking water with much lower detection limits. The aims of this study are to validate our new method for the analysis of poly- and perfluoroalkyl substances (PFAS) in drinking water and to test its feasibility by analyzing PFAS in drinking water from Northern California. Our new method for water PFAS analysis builds upon the USEPA method 537 (solid phase extraction and liquid chromatography/tandem mass spectrometry) and expands the list of PFASs to include: eight perfluoralkyl carboxylic acids (PFCAs), four perfluoroalkane sulfonic acids (PFSAs), two perfluoralkyl phosphonic acids (PFPAs), three perfluoralkyl phosphinic acids (PFPiAs), eleven PFCA precursors/intermediates, and three PFSA precursors. We use a WAX SPE cartridge (Oasis, Waters) for concentration and a 9 minute analysis using liquid chromatography (Nexera UFLC system, Shimadzu) coupled to a triple-quadrupole tandem mass spectrometer (SCIEX QTRAP 5500 MS/MS system). The instrument detection limits range from 1.0 to 480 ng/L, with detection limits of 1.2 ng/L and 6.2 ng/L for PFOS and PFOA, respectively, which are sufficiently sensitive to comply with the new USEPA Health Advisory Guideline. Disclaimer: The views expressed herein are those of the authors and do not necessarily reflect those of the California Department of Toxic Substances Control.

TP153 Need for Data on Environmental Fate of Perfluoro Ethers

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Perfluoro ethers (PFEs) are widely used in a variety of applications including electronic, aerospace and high temperature lubricants, membranes in fuel cells, and consumer applications. They are also increasingly being used as replacements for perfluoro carboxylic acids (PFCAs) and sulfonic acids (PFSAs) in these and other applications. Perfluoro ethers are released to the outdoor and indoor environment, and have recently been detected in water and biota. Understanding the fate and exposure of PFEs are required to evaluate risk to human and non-human species. USEPA has attempted to determine the fate of PFCs as part of an evaluation of potential risk for a number of perfluoro ethers using standard New Chemical assessment approaches. Review of the environmental and industrial chemistry literature and confidential literature submitted to the New Chemicals program by EPA found only very limited information on PFE degradation. PFEs are reported to degrade in laboratory studies at elevated temperatures and in the presence of photocatalysts, strong Lewis acids, hydroxyl and other radicals suggesting that the ether linkage can be degraded to form smaller compounds, shorter poly ethers and PFCAs. It is unknown if these processes occur in the environment, at what rates and under what conditions. Information on the environmental fate of PFCs, needed to be able to evaluate their fate under environmental condition, does not exist. Studies of abiotic degradation including hydrolysis, direct and indirect photolysis, catalysis by dissolved species and on geologic surfaces are needed to determine fate and transport properties of PFEs. Biological transformation data are also needed including data on aerobic and anaerobic microbial degradation in water wastewater sediment, sludge, and soil. These studies should provide sufficient information to determine environmental degradate rates, mechanisms and pathways to better understand exposure to humans and environmental species. Until this information is available, significant uncertainty will be associated with estimates of potential risk of perfluoro ethers. The views expressed in this abstract are those of the authors and do not represent Agency policy or endorsement.

TP154 Accumulation Potentials of Perfluoroalkyl Acids (PFAAs) in Crops Irrigated With Reclaimed Water

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Significant contamination of perfluoroalky acids (PFAAs) in wastewater treatment plants (WWTPs) implicates that the reuse of treated wastewater for agricultural irrigation and the practice of applying treated sludge as a potential source of these contaminants onto agricultural farmlands. The aim of the present study was, therefore, to determine the accumulation potential of perfluoroalkyl carboxylic acids (PFCAs) and perfluoroalkyl sulfonic acids (PFSAs) in crops irrigated with treated wastewater under field conditions. Reclaimed water with or without a fortification of PFAAs was used to irrigate crops until harvest. Results from the present study will enhance the understanding on the accumulation of PFAAs in crops irrigated with treated wastewater under field conditions.

TP155 Transformation of poly- and perfluoroalkyl substances (PFAS) in petroleum hydrocarbon-contaminated soil during biopile treatment

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Aqueous film-forming foam (AFFF) formulations that contain poly- and perfluoroalkyl substances (PFAS) are used to extinguish hydrocarbon-fuel fires for their excellent performance in lowering surface tension at the air-AFFF interface. PFAS were detected at ppb to ppm levels in soil biopiles for petroleum hydrocarbon bioremediation. The PFAS presence was the result of the AFFF release for fire-fighting at an oil leakage site. Under the biopiling conditions when petroleum hydrocarbons biodegradation are augmented, some PFAS with hydrocarbon functionalities may be biotransformed to produce persistent perfluoroalkyl acids such as perflurooctane sulfonic (PFOS) and carboxylic acids (PFOA). PFOS and PFOA are subject to stringent regulations due to their toxicity and persistence. Therefore, it is essential to understand the environmental fate of PFAS in soils co-contaminated with petroleum hydrocarbons and AFFF. The main objective of this research is to examine, under biopile treatment conditions when petroleum hydrocarbons are biodegraded, possible biotransformation of the PFAS present in a field-contaminated soil. Contaminated soils from bioplies with and without nutrient (e.g., N, P, K, micronutrients and organic matter) amendment were incubated aerobically for 3 months. The levels of petroleum hydrocarbon and PFAS were monitored. Petroleum hydrocarbons of Fraction 2 and 3 were solvent extracted and analyzed by GC-FID. A total of 34 PFASs were monitored using LC-MS/MS, including PFOS, PFOA and their homologues, fluorotelomer sulfonates and fluorotelomer betaines. 6:2 fluorotelomer sulfonamide alkylbetaine (6:2 FTAB), a major constituent of AFFF formulation, was also spiked to the same soil to examine for its possible biotransformation under the similar conditions with three different nutrition levels. Biotransformation kinetics of the petroleum hydrocarbons and 6:2 FTAB, formation yeilds of high-priority PFAS, and the impact of nutrient amendment will be presented and discussed. It is expected that this research would help us understand the behaviour of PFAS in soil co-contaminated with petroleum hydrocarbons thus to better manage such contaminated sites.

TP156 Perfluoroalkyl acid tissue distribution in healthy and pansteatitis-affected Tilapia (Oreochromis mossambicus) at Loskop Dam, South Africa

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Perfluoroalkyl acids (PFAAs) are anthropogenic contaminants of concern that are found predominantly in blood and blood-rich organs (e.g., kidney and liver) largely because PFAAs are predisposed to associating with blood proteins like albumin. Measuring PFAAs in blood or plasma is advantageous as it provides a medium to asses PFAA levels without animal sacrifice. Thus, many experimental designs measure blood or plasma levels of PFAAs in studies where sacrifice is not preferred or possible. However, the health status of an individual can alter the level of protein and albumin in the blood (e.g., inflammation) and could impact the levels of proteins available to associate with PFAAs. Under this premise, it is unknown whether the PFAAs that can no longer bind to albumin remain in the blood rich matrices, are sequestered in alternate organs, or are excreted. A population of Mozambique tilapia (Oreochromis mossambicus) at Loskop Dam, South Africa, has been suffering from an inflammatory disease known as pansteatitis for many years. Preliminary investigations of PFAA plasma levels in this population of pansteatitis-affected tilapia have shown significantly lower levels of both albumin and PFAAs in the diseased tilapia plasma compared to healthy tilapia plasma. In this study, we investigate the possibility that disease and inflammation in this population of tilapia could alter the PFAA tissue distribution compared to healthy tilapia, resulting in potential wider implications in the understanding of PFAA burden as it relates to disease for the perfluorinated scientific research community.

TP157 Perfluoroalkyl phosphinic acids form the corresponding phosphonic acids and 1H-perfluoroalkanes in rats

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Perfluoroalkyl acids (PFAAs) are a class of widespread contaminants that are generally accepted as recalcitrant under environmental conditions. A 2012 paper from our group showed evidence of the first biotransformation of any PFAA, where perfluoroalkyl phosphinic acids (PFPiAs, $(F(CF_2)_x)(F(CF_2)_y)PO_2^-$) were metabolically hydrolyzed to perfluoroalkyl phosphonic acids (PFPAs, $F(CF_2)_xPO_3^{2-}$) in fish. PFPiAs and PFPAs are found together in industrial mixtures that have been used directly as surfactants for both agricultural and industrial applications. To confirm the in vivo transformation in mammals and identify the remaining PFPiA metabolite, rats were dosed with 50 µg/kg of either C6/C8 PFPiA, C8/C8 PFPiA, or C8 PFPA via oral gavage. Blood, kidneys and liver were sampled to determine pharmacokinetic and partitioning properties. PFPAs and PFPiAs were quantified via UPLC-MS/MS using matrix matched calibration. An additional group of rats was dosed at 2 mg/kg to probe the formation of volatile products.1H-perfluoroalkane metabolites were detected in blood using a novel headspace solid phase microextraction method with negative chemical ionization GC/MS. Both PFPiAs were hydrolyzed in vivo to form PFPAs and 1H-perfluoroalkanes. 1H-perfluoroalkanes were not detected in rats dosed with C8 PFPA, suggesting the C-P bond cleavage requires the presence of a second electron withdrawing perfluoroalkyl group. Although metabolism decreases the concentration of PFPiAs, the products formed have the potential to be more persistent and toxic. PFPAs have been shown to be persistent in the literature and in this work, while shorter 1H-perfluoroalkanes have been shown to form reactive acid fluoride intermediates that covalently bind to proteins. In vitro experiments using rat subcellular liver S9 fraction were performed to further investigate the metabolism of PFPiAs. This work shows the first detected mammalian hydrolysis of C-P bonds in phosphinates and the first confirmation of 1H-perfluoroalkanes as metabolites of PFPiAs.

TP158 Endocrine-disruption potential of perfluoroethylcyclohexane sulfonate (PFECHS) in chronically exposed Daphnia magna

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Perfluoroethylcyclohexane sulfonate (PFECHS), mainly used in hydraulic fluids in aircrafts, is a member of the perfluoroalkyl sulfonate family which includes the regulated perfluorooctane sulfonate (PFOS). PFECHS has been reported in environmental samples but its toxicity to aquatic organisms is unknown. The objectives of this study were to identify biological pathways impacted by sublethal exposure (12d) of D. magna to PFECHS (0.06, 0.6, and 6 mg/L) using microarray and quantitative real-time PCR and to develop a relevant biomarker to link transcriptomic to phenotypic responses. PFECHS was also quantified in surface water samples (1.04 to 1.38 ng/L)collected from the St. Lawrence River, Canada. All transcriptomic analyses indicated the under-regulation of vitellogenin-related genes (VTG1 and VTG2)in treatment groups. PFECHS exposure also led to the up-regulation of genes related to cuticle. VTG was selected as a cellular marker and identified in D. magna using an immuno-specific assay and quantified using Western Blot and LC/MS/MS. Results indicated a decrease of VTG content in exposed D. magna which was in concordance with the transcription of VTG-related genes. No impacts were observed on survival, molting, or reproduction at the individual/population levels. Overall, results suggest endocrine disruption potential for PFECHS in these invertebrates.

TP159 Sources of human exposure to poly- and perfluoroalkyl substances (PFASs)

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Human exposure to poly- and perfluoroalkyl substances (PFASs) has been linked to a variety of adverse health effects including cancer, elevated cholesterol, and disrupted immune and endocrine systems. Consumer products, drinking water and marine foods are all sources of exposure to PFASs. Those with environmentally mediated pathways (drinking water and marine foods) respond to regulations over different time scales than consumer sources, which can be altered quickly by changes in manufacturing. Most available exposure source attribution for PFASs is based on estimated chemical doses from different sources (external doses) without validation against exposure biomarker concentrations. Many of the parameters involved in this process such as exposure frequencies and pharmacokinetic parameters are hard to measure, resulting in large uncertainties in the exposure estimates. Here we use measured serum concentrations for 11 frequently detected PFASs homologues and a chemical fingerprinting technique to refine understanding of exposure sources. Data are from multiple cohorts representing diverse exposure pathways in the Faroe Islands, Vancouver, Canada and the US National Health and Nutrition Examination Survey, collected around the same time. We found the homologue profiles from a seafood consuming population in the Faroe Islands are distinctly different from the one in Vancouver where exposures from consumer products may be more dominant. We quantify the impact of exposure frequencies (such as frequency of marine food consumption and consumer good exposure) on serum PFASs homologue composition to identify the PFASs that best represent each exposure source. We use this analysis to better understand the relative contribution of these two sources to PFAS exposures in the US general population.

TP160 Perfluoroalkyl acids among Korean children and adolescents of 4 to 18 years of age: serum levels, related sources, and health outcomes

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Perfluoroalkyl acids (PFAAs) have been frequently detected in humans. However, their exposure among children and adolescents and associated health effects are not well understood. In this study, 16 perfluoroalkyl acids (PFAAs) were analyzed from a total of 150 serum samples collected from 4-18-year-old children and adolescents of Seoul and Gyeonggi, Korea, in 2012 and 2014. Among the target PFAAs, PFOA, PFNA, PFHxS, PFOS, and PFUnDA were detected in >98% of the samples. PFOS showed the highest detected concentration with a median concentration of 5.68 ng/mL, followed by PFOA, PFNA, PFHxA, and PFUnDA. Detection rates of PFDA and PFDS were between 60% and 80%, while the other PFAAs were detected in less than 50% of the samples. Among 16-18-year-old adolescents, total PFAAs concentration in serum was significantly higher among males than in females. However, this gender difference was not significant in the other age groups. Concentrations of several PFAAs, i.e. PFOA, PFNA, and PFOS, decreased as age increased. Using questionnaire data, several dietary and behavioral factors, i.e. breastfeeding, fish/shellfish consumption, use of frying pan, and use of waterproof coating cloths, were identified as potential exposure sources of PFAAs. Among the target PFAAs, PFUnDA showed significant positive association with total cholesterol, low-density lipoprotein cholesterol, or fasting blood glucose. The effects of PFAAs on cholesterols warrant confirmation in other juvenile populations and should be tested in animal experiments.

TP161 Using Cats as Model for indoor Exposure – PFAS and Association to Feline Thyroidogenicity

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Thyroidogenic disruption as an endocrine endpoint is of growing concern for human health and the environment. Hence, the MiSSE project (Mixture aSSessment of EDCs) is specifically aiming to assess the indoor exposure situation to thyroid hormone disrupting compounds (THDCs), and accordingly the mixture effects of these compounds. As cats and toddlers have a similar behavior with their grooming and hand-to mouth activity cats are here used as sentinels for human and child exposures to indoor related chemicals and their thyroidogenic effects. Recently, an association was demonstrated between domestic cat's thyroid health status and levels of brominated flame retardants in their blood (Norrgran et al 2015, ES&T 49:5107). We have further investigated the exposure pathway via dust by correlating the levels in the two matrices dust and cat blood. The THDCs are searched for by applying in silico similarity models comparing known THDCs (Zhang et al 2015, ES&T 49:10099; Weiss et al 2015, ABC 407:5625) and using target analysis of a selection of key-THDCs. Dust and cat serum samples have been sampled in the Stockholm/Uppsala region, Sweden between 2013-2014. The families had healthy pet cats and at least one child living at home (< 10 years old). Dust samples were vacuumed from the living room and bedrooms of the participating families. Still-standing dust were collected using a dust collector ((Dustream™, Indoor Biotechnologies Ltd., Wiltshire, UK) containing a disposable filter (mesh size 40 µm) attached to a household vacuum cleaner tube. The dust was collected from surfaces little influenced by daily life e.g. walking and/or containing bread crumbs and soil. The samples were sieved (1 mm) to isolate the estimated ingested fraction for cats (0.04-1.0 mm). The target analytes, e.g. brominated flame retardants, organophosphorus compounds, organochlorines, perfluoroalkyl substances (PFAS) and some phenolic compounds have been determined with GC and LC-MS/MS instrumentations. Statistical correlations between cat serum and house dust in paired samples have been assessed. In addition, an Effect-Directed Analysis approach utilizing advanced chemical instrumentation and bioassay guided fractionation will be performed on the household dust in the strive to identify key-THDC. We will focus the presnetation on the PFAS analysis specifically but also the current status of the project, focusing on dust and cat serum contaminants, mixture effects and evaluation of the indoor exposure to a set of emerging compounds of concern.

TP162 Addition of metals to enhance abiotic transformation of perfluorooctane sulfonic acid in vitamin B12 and permanganate systems

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The use of adding nano(n)- or micro(m)-sized zero valent metals to enhance the potential of vitamin B_{12} (B12) and permanganate (PM) in transformation of perfluorooctane sulfonic acid (PFOS) was evaluated in aqueous batch systems. In the B12 systems in which nFe^0 and nZn^0 were evaluated with temperature and over time, loss of linear (L-) and branched (Br-) PFOS isomers were quantified using time of flight (TOF)/MS/MS and generation of F^- and SO_4^{2-} byproducts were monitored using ion chromatography. For identification/exploration of polyfluoroalkyl intermediates, the aqueous phase, meal extracts and headspace were analyzed using TOF/MS/MS and GC-ECD/MS, respectively. In B12 with nFe^0 at pH 10.4 and 21 d, increasing temperature from 22 to 70 °C increased the degree of PFOS removal (20 ± 2 % at 22 °C and 32 ± 5 % at 70 °C) with 1 ± 0.1 and 9 ± 1 moles of F^- for every mole of PFOS removed was observed at 22 and 70 °C, respectively. In addition, 0.8 ± 0.1 mole of SO_4^{2-} per mole of PFOS lost was detected at 70 °C. In B12 with nZn^0 at pH 10.4 and 21 d, 22 ± 3 % of PFOS loss with 7 ± 1 moles of F^- generated per mole of PFOS lost and 34 ± 3 % of PFOS loss with 19 ± 2 moles of F^- generated per mole of PFOS lost was observed at 22 and 90 °C, respectively. The estimated first-order removal rates of the branched PFOS isomers were 2.9, 1.4, and 0.3/d for 6-PFOS, 3&4-PFOS and 5-PFOS, respectively at 90 °C in the B12-nZn^0 system. No loss of L-PFOS was observed, thus limiting B12 technologies considering that L-PFOS is the most prevalent isomer in groundwater. This led to exploring enhancing the PM system, which has been shown to have potential to degrade PFOS. The addition of mMg^0 and mZn^0 as catalysts to PM solutions at 65 °C and pH 6~7 (12 d) enhanced PFOS removal to 35 ± 6 % and 29 ± 0.1 %, respectively, compared to only 11 ± 2 % in PM alone. The PFOS lost included Br- and L-PFOS unlike the B12 system. F^- generation was quantified in the aqueous phase and observed in all three systems but appeared inversely correlated to the % PFOS lost with 9 moles of F^- per mole of PFOS lost in PM alone and only ~ 4 and ~ 2 moles of F^-/PFOS lost with mZn^0 and mMg^0, respectively. Additional F^- was likely generated, but readily adsorbed to the metal catalysts. Extraction of adsorbed F^- and SO_4^{2-} is underway as well as a more detailed characterization of kinetics, organic metabolites, and mechanisms in the PM system.

TP163 Reduction of perfluorooctanesulfonate (PFOS) and perfluorohexanesulfonate (PFHxS) by synthesized activated carbon-supported bimetals

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Perfluoroalkyl substances (PFASs) have been detected in various environmental and biological matrices including groundwater. Perfluorooctanesulfonate (PFOS) and perfluorohexanesulfonate (PFHxS) are stable and resistant to typical environmental degradation processes. Here we examine the degradation performance of PFOS and PFHxS by activated carbon (AC) supported bimetallic nanoparticles (NPs). Bimetallic NPs included a combination of Ni, Fe, Cu, Pd, Ce, Sr, and Mg, which were synthesized onto AC. Various approaches for enhancing reactivity of the bimetallic catalyst were investigated, including increasing temperature (60°C) and adding a third metal/catalyst or magnetite (Fe_3O_4) as well as a combining reduction-oxidation processes by addition of persulfate. Batch reactions were carried out for 5 days in triplicates with 3 mg/L of PFOS and PFHxS. Degradation of PFOS and PFHxS was quantified in the aqueous and in solvent extracts after a series of exhaustive sequential extraction. F^- and SO_4^{2-} were identified using ion chromatography and organic byproducts using LC/MS/TOF. Among the several bi/trimetal systems probed, the NiFe° bimetal system plated on an AC support at 60°C resulted in the greatest loss of PFOS (~35% in 5 d). PFOS removal was accompanied by the release of the sulfate ion (SO_4^{2-}) in $NiFe^0$,Fe^0-AC and $NiFe^0$-AC samples (~0.33, ~0.30, and ~1.08 moles of sulfate per mol of PFOS removed, respectively). Also in all samples that showed PFOS loss, pH values increased to ~9. Fluoride could not be quantified due to the presence of an unknown peak in the AC systems. The addition of Fe_3O_4 to the NiFe-AC did not enhance or reduce reactivity towards PFOS; however, its presence may serve to aid in degradation of co-contaminants in mixed-contaminant systems. The addition of a third catalyst to the NiFe-AC system did not increase PFOS removal and the addition of persulfate decreased PFOS removal. No clear trend in PFOS loss with the reduction potential of the various metal catalysts was apparent suggesting that catalyst selectivity rather reduction potential is controlling PFOS removal.

Modeling and Monitoring of Environmental Pesticides Exposure: Regulatory Context and Improvements from Science

TP164 Impacts of Seawater on Photoproduct Formation of Dichloran

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The salinity of seawater can influence both the overall rate of degradation of chemicals and impact the distribution and types of photoproducts from the photodegradation of a pesticide. The fungicide dicloran, 2,6-dichloro-4-nitroaniline (DCNA), is applied to crops grown in areas near both freshwater and saltwater bodies where it is susceptible to photolysis; limited information is published on the photodegradation of DCNA. The photodegradation of DCNA was measured in distilled water, artificial seawater, estuarine water, and phosphate buffer to determine the degree of differences in the degradation rate and product distribution of the compound in various matrices. Solutions of DCNA at a concentration of 1 ppm were prepared and irradiated for 24 hours in an Atlas SUNTEST XXL+ photochamber that mimics the wavelength distribution and intensity of sunlight. Samples were withdrawn at 0, 2, 4, 6, 12, and 24 hours and analyzed for residual DCNA using an Agilent 1260 Infinity High Performance Liquid Chromatograph. The formation of ions such as nitrate, nitrite, bromide, and chloride were measured using a Thermo Dionex ICS-5000+ Ion Chromatograph. The half-life of DCNA in distilled water was calculated to be 7.62 ± 0.094 hours and 7.37 ± 0.279 hours in artificial seawater; statistically there was no significant difference in the degradation rate. Analysis of the quick formation of nitrite and chloride ions, and later formation of nitrate ions, suggests photonucleophilic substitution processes are occurring as the compound is degrading, followed by further degradation of nitrite to nitrate likely also due to photolysis processes. Measurable intermediate photoproducts were analyzed for formation rate; a difference in the concentration of product was noted between distilled water and artificial seawater suggesting salinity affects the rate of formation of this photoproduct.

TP165 Photo-induced Toxicological Effects of 2,6-dichloro-4-nitroaniline (DCNA) on P. promelas (Fathead Minnow)

E.N. Vebrosky, Louisiana State Univ / Dept of Environmental Sciences; K. Armbrust, Louisiana State Univ / Environmental Sciences School of the Coast and Environment

Toxic effects of chemicals can be initiated after that chemical has been exposed to light. The fungicide 2,6-dichloro-4-nitroaniline (DCNA) is applied to many crops that are grown in areas where agricultural runoff enters bodies of water. DCNA is susceptible to photolysis as it enters the surface layer of water bodies, therefore increasing the chances of toxicological effects on various aquatic species that may be exposed to the chemical itself as well as the intermediate photodegradation products. This can be especially relevant in shallow water aquatic system such as salt marshes and estuaries. Previous research on PAHs has shown increased toxicological effects to species exposed to both the chemical and sunlight (or UV light). Many of these effects seem to be related to quinone intermediates generating intercellular oxidant ultimately resulting in cell damage. DCNA appears to photo-degrade through quinone-hydroquinone intermediates, thus juvenile fish or aquatic invertebrates may be especially susceptible to phototoxic impacts from exposure to this chemical.

TP166 Determination of Physicochemical Properties of Atrzanie, Fipronil and Difficult-to-Test Substances: Pyrethroid Pesticides, in Freshwater and Seawater

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Salinity has been reported to impact the water solubility of organic chemicals entering marine ecosystems however, there is scarce data available on salinity impacts for pesticides potentially entering seawater. Impacts on water solubility would corresponding impact chemical sorption as well as overall bioavailability and corresponding exposure estimates used in the regulatory assessment of pesticides. The insecticides fipronil, bifenthrin, cypermethrin and the herbicide atrazine are used heavily worldwide and can potentially be transported to water bodies through runoff or leaching. The water solubility and n-octanol/water partition coefficient (log K_{ow}) of atrazine and fipronil were determined in distilled water and artificial seawater using the OECD 105, flask method, and OECD 107, shake flask method, respectively. The water solubility and log K_{ow} of pyrethroids were determined in distilled water and artificial seawater using the OECD 105, column elution method, and the OECD 123, slow stir method, respectively. The water solubility of atrazine, fipronil, cypermethrin (a mixture of eight isomers), and bifenthrin were 56 ± 1, 4.8 ± 0.96, 0.78 ± 0.1, and 0.1 ± 0 in distilled water, versus 45 ± 0.24, 1.9 ± 0.25, 0.08 ± 0.02, and 0.04 ± 0.01, in artificial seawater. The log K_{ow} of atrazine, fipronil, cypermethrin, and bifenthrin were 2.55 ± 0.07, 3.48 ± 0.22, 5.53, and 6.63 in distilled water, versus 2.74 ± 0.09, 4.24 ± 0.35, 7.86, and 7.05 in artificial seawater, respectively. To date the log K_{ow} values of the pyrethroids in distilled water and artificial seawater were estimated from their water solubility. Overall, the water solubility of pesticides were decreased in artificial seawater versus distilled water while the log K_{ow} of pesticides were increased in artificial seawater versus distilled water. Salinity appears to generally decrease the water solubility and increase partitioning potential, as indicated by the log K_{ow}. Dramatic differences in chemical partitioning behavior in seawater versus freshwater suggest salinity should be taken into account when conducting exposure estimates for marine organisms.

TP167 A Spatially-distributed Modeling Framework to Integrate Effects of Agricultural Best Management Practices: A Midwest Case Study

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The EPA pesticide risk assessment framework does not currently quantitatively account for off-field transport and exposure effects of best management practices (BMPs). Within the agricultural landscapes of the U.S., numerous structural and cultural BMPs are currently in place, mostly developed from NRCS programs to address agrichemical and soil loss reduction, natural resource conservation, and yield increase. In this work, a Midwest watershed was used as a case study for development of a spatially-distributed modeling approach to quantitatively evaluate potential impact of pesticide reduction (load and/or concentration) from multiple BMPs at multiple scales. This framework included a hydrologic and chemical transport model that was paired with stream flow and routing components to represent upland hydrologic processes, land management practices, and a flowing, receiving water body. Within the upland model, specific sub-areas were modeled separately to represent various structural BMPs, based on their physical location. Furthermore, cultural BMPs (non-structural such as application type, rate, and timing) were also incorporated into the upland model to different degrees to evaluate model sensitivity. A baseline scenario was developed and used to define comparative difference between addition (adoption) and elimination of BMPs in a step-wise fashion. As part of the baseline scenario, relevant literature values and publically-available environmental data were used to inform model parameterization. This modeling framework can be used to explore how BMPs may be considered in a regulatory framework, addressing issues such as: BMP adoption rate and viability, weather-dependent behavior, and frequency and extent of BMP efficacy.

TP168 Advances in the Refined National-Scale Drinking Water Assessment Framework: Case Study Chlorpyrifos

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In the US pesticide regulatory scheme for human dietary exposure, estimates of the surface-derived drinking water contribution are assessed with simple scenario-based modeling techniques. Such estimates can be a useful and conservative approach in a tiered risk assessment to systematically eliminate potential concern; however, the limited conclusions that can be reached are often not adequate to address the true extent of potential exposures. The current work focuses on advances in national-scale drinking water concentration estimation techniques, using chlorpyrifos as a case study. The method incorporates spatially-distributed environmental and management information in a risk framework at the watershed (HUC12) scale. Concepts of standard scenarios to create conservative, regionally representative (at an extent wider than HUC12) estimates of exposure patterns were applied and then were distributed at the HUC12 watershed scale by matching them with HUC12-specific cropping patterns derived from five years of cropland data layer (CDL) data. As a conservative first approximation, it was assumed that a drinking water source co-occurs within every HUC12 where applications were made to labeled crops. In this way, HUC12-specific exposure estimates were derived for all chlorpyrifos-labeled crops. Then, county-level community water system (CWS) locations were included to eliminate risk concern from HUC12 watersheds that did not co-occur with a CWS. Results from this first approximation eliminated concern in 83% of HUC12 watersheds with labeled crop(s). The impact of refinements and assumptions on the remaining 17% of HUC12 watersheds was further explored. Potential refinements included higher-resolution rainfall, soils, runoff and erosion data inputs, more realistic environmental fate, application timing and application type information, and percent crop treated. Significant impact was observed from the inclusion of these refinements, with at least 92% of the remaining HUC12 watersheds yielding a conclusion of no concern. Characterization of potential risk in a spatially-distributed fashion increases risk characterization accuracy/certainty because of the inclusion of best-available data to inform estimates of exposure and co-occurrence. In conclusion, it was discovered that spatially-distributed environmental and management factors must be considered to increase certainty and characterize potential risk, rather than relying solely on a scenario-based approach.

TP169 Current Pesticide Regulations Do Not Protect Global Surface Waters

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Current high-intensity agricultural practices lead to serious consequences for the global environment. Pesticides, in particular, are highly biologically active substances that can threaten the ecological integrity of nontarget ecosystems such as surface waters. However, considering particularly larger scales, our field-data based knowledge regarding the pesticide exposure of aquatic ecosystems is very restricted. The current study evaluated based on a comprehensive meta-analysis of 838 scientific studies the insecticide exposure of global agricultural surface waters (>2,500 sites in 73 countries). This analysis tested whether insecticide field concentrations (water and sediment) exceed legally accepted regulatory threshold levels (RTLs) derived from official EU and US pesticide registration documents and, amongst others, how risks depend on insecticide development over time and stringency of environmental regulation. The most important result of this study is that 52.4% out of 11,300 insecticide field concentrations exceeded respective RTLs. This finding indicates a substantial risk for the biological integrity of global water resources as we show that the regional aquatic biodiversity is reduced by approximately 30% at pesticide concentrations equaling the RTLs. Further analyses prove that RTL exceedances are significantly higher for newer-generation insecticides (i.e., pyrethroids) and are high even in countries with stringent pesticide regulations (e.g., United States; EU member countries). The overall findings of this meta-analysis substantiate that agricultural insecticides threaten surface waters globally and that the current pesticide regulatory risk assessment approaches fail to protect the aquatic environment.

TP170 Modeling spray drift and runoff related inputs of pesticides to receiving water

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Pesticides move to surface water via various pathways including surface runoff, spray drift and subsurface flow. Surface runoff is often considered as the most important transport route in many areas. However, in some watersheds, spray drift has been shown to be a significant contributor. Little is known about the relative contributions of surface runoff and spray drift in agricultural watersheds. This study attempts to develop a modeling framework to address the contribution of spray drift to the total loadings of pesticides in receiving water bodies. The modeling framework will consist of models for simulating spray drift, surface and subsurface runoff and environmental fate processes in receiving water. Monitoring data collected from daily samples were used for model evaluation. Preliminary results showed good agreement between model predicted concentrations for drift events and less so for runoff events. Once validated, the modeling framework could be a valuable tool to the decision making in pesticide risk management and mitigation such as where and what types of best management practices to implement in a watershed.

TP171 Deposition and spray drift ratio of commercially used sprayers

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Optimal pesticide spray deposition is essential in order to achieve effective pest, weed and disease management. Differing designs of commercially available sprayers can possibly increase deposition especially as crop type, canopy density, and geographic features of the field change. Most sprayers can be adjusted to maximize application efficiency. Common changes include nozzle size, vertical orientation of nozzles, and fan speed. However, these adjustments may not completely eliminate spray drift onto non-target areas which can increase the environmental — bees, parasitoids, predators and aquatic and terrestrial organisms— and farm-worker risk. We evaluated three different spray technologies— airblast, cannon, and electrostatic technologies—in order to create comparable baseline knowledge regarding canopy deposition, aerial drift and mid-row floor deposition. Spray applications were conducted using a tank mix containing water-soluble Pyranine 10G® fluorescent tracer. Air and ground drift samplers were placed above the canopy zone and on the ground in adjacent rows to measure the respective levels of fluorescent spray deposition. Replicated samples were collected, refrigerated, and subsequently measured by using fluorometry technique. Preliminary results showed that electrostatic sprayer had increased in-canopy coverage and lower levels of aerial drift. The cannon sprayer displayed lower levels of ground deposition compared to electrostatic and airblast sprayers. These results indicate both advantages and drawbacks of the respective tested sprayers and may help growers make more informed decisions regarding optimal application technologies for their field and the environment.

TP172 Importance of Time-Dependent Sorption in Ground Water Modeling in Regulatory Assessments

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Scientists have recognized for over 30 years that sorption of crop protection products and their metabolites increase with time. This process is typically referred to as time-dependent sorption (TDS), aged sorption, or kinetic sorption. From a regulatory viewpoint, these effects are especially important for compounds with moderate sorption when assessing potential movement to ground water, but in some cases the effect of increasing sorption with time can be a critical factor for quite mobile compounds. This will be illustrated with examples comparing monitoring data with modeling including and not including TDS. Currently TDS is generally modeled considering a liquid phase and two soil phases, one in equilibrium with the liquid phase and the other with sorption and desorption with the liquid phase described kinetically. Incorporation of TDS in the registration process has been discussed in Europe since 1997 and guidance first issued in 2000. The ability to model TDS was introduced in 2000 in the regulatory models used in the European Union for assessing movement to ground water, but discussions on guidelines for experimental studies and the derivation of model parameters are still continuing. In the United States, use of TDS for regulatory applications has been delayed as discussions continue on the appropriateness of describing of the process using a liquid phase and two solid phases.

TP173 Use of a tiered modeling approach for EU GW modeling

P. Sharma

The EU approval and national authorisation processes under Regulation (EC) 1107/2009 require the assessment of the potential of an active ingredient and its metabolites to move to ground water (FOCUS, 2015). FOrum of Coordinated USe of Pesticides (FOCUS, 2015) presents a standard set of scenarios and models that can be used to assess the potential movement of pesticides and their metabolites with soil leachate under a broad range of environmental conditions in Europe. The scenarios are intended to be broadly representative of a wide range of European agricultural settings vulnerable to leaching. The FOCUS guidance offers a tiered structure to groundwater modeling with conservative inputs and approaches at the lower tier and possibility of refinements at the higher tier. Refinements with field data, plant uptake factors, inverse modeling and use of aged sorption parameters can be considered as higher tier approaches. For this study, the leaching behavior of dummy substances provided in FOCUS,2015 were assessed by means of simulation runs with the leaching model FOCUS PEARL 4.4.4. Tomato was used as an example crop for simulations. The following tiered approach was used: 1) tier 1 gw modeling – lab degradation endpoints, 2) tier 2 – field degradation parameters 3) field data with plant uptake factor 4) field data, default plant uptake factor and default aged sorption parameters from FOCUS, 2015 5) default plant uptake and variation of default aged sorption parameters from FOCUS, 2015 gw guidance. Results were compared for all the tiers modelled. The simulations indicate that predicted environmental concentrations in groundwater for active substances and/or metabolites can vary by up to 3-5 times depending with parameter refinements and higher tier modeling approaches.

TP174 Use of laboratory determined subsoil sorption data to refine EU ground water leaching assessments

F.P. Donaldson, BASF Corporation / APDEFR

In European regulatory leaching assessments, the predicted concentrations of pesticides and their degradation products in ground water are highly dependent on soil adsorption input values. Modeling results are particularly sensitive for low sorbing compounds, e.g. those with organic-carbon normalized sorption coefficients ($K_{f,oc}$) of < 100 mL g^{-1}. Demonstrating that sorption is not correlated with soil organic carbon can be difficult with low sorbing compounds, leading to the implementation of a $K_{f,oc}$ in ground water leaching models and often exceedances of the pertinent ground water limit (e.g. 0.1 ug L^{-1}) for parent and relevant metabolites. Studies investigating sorption to subsoils, particularly those of low organic carbon, may demonstrate that sorption occurs both independently of organic carbon and throughout the soil profile. Such studies could thus lead to refinement of the sorption parameter for ground water modeling and more realistic estimates of ground water leaching potential. A refinement of the sorption endpoint for ground water modeling, based on subsoil sorption studies, will be presented and discussed. In particular, a high speed centrifugation plus filtration technique to enable accurate determination of sorption values for low sorbing compounds will be presented. The resulting subsoil sorption endpoints will be compared with topsoil sorption endpoints, and their implementation in ground water leaching models will be compared against the more conservative $K_{f,oc}$ approach.

TP175 Use of Pesticides Groundwater Monitoring data as higher tier for regulatory risk assessment – Feedbacks from France

A. Boivin, ANSES

Pesticides risk assessment in the EU regulatory framework is mandatory performed for pesticides. In both cases, no unacceptable risk of groundwater contamination has to be proven. Routinely, Predicted Environmental Concentrations in GroundWater (PECgw) are derived using numerical models for pesticides and metabolites. In addition to modelling, the European regulatory framework also allows for Groundwater Monitoring data to be used for pesticides approval. In the tiered approach, Groundwater Monitoring data are identified as higher tier that may supersede the modelling. Still, there is currently no European guidance document available on the use of groundwater monitoring data for regulatory purposes. In France, Groundwater Monitoring data have been submitted to Anses (French Agency for Food, Environmental and Occupational Health & Safety) in order to support the renewal of pesticides. The aim of this presentation is to provide a feedback based on different worked examples. These feedbacks include focus on the main issues that were identified and the general recommendations that were made when targeted monitoring were submitted. The main issues when dealing with Groundwater Monitoring data interpretation were related to site selections and related vulnerably, and then of how representative was the GroundWater monitoring. Recommendations were made to address both issues and will be presented. In addition, proposal to combine targeted together with public Groundwater Monitoring dataset were made to enhance the representativeness of the GW monitoring conducted and will also be presented.

Modeling Human and Ecological Exposure over the Life-Cycle of Consumer Products

TP176 Aqueous leaching of chemical additives from microplastics

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Plastics are ubiquitous in our daily lives; where they constitute major components of materials in our homes, workplaces, hospitals, schools & vehicles. Our food, beverages & medicines are stored in plastic containers. Most plastics are eventually discarded & enter terrestrial & aquatic environments in massive quantities. They fragment over time into microplastics, which exhibit greatly enhanced surface areas & reactivities. Once released, plastics may impact health & ecological processes. As plastics are compositionally diverse & exhibit differing properties, treating them as a homogenous class in terms of fate & health impacts is inappropriate. A multitude of additives are also incorporated into polymers, often at % by weight levels, to alter their appearance, flame retardancy, flexibility & other properties. The additives themselves exhibit varying behaviors & toxicities. While it was long assumed that additives remained within polymers indefinitely & thus exposure risks were minimal, this is untrue. For example, humans & wildlife contain substantial burdens of additives, such as persistent, lipophilic brominated flame retardants (BFRs). Approaches to systematically evaluate the mobility of additives from polymers under a wide array of conditions are needed. We examined here the ability of aqueous fluids of varying temperature & humic acid composition to leach additives from polyurethane foam (PUF), acrylonitrile butadiene styrene (ABS), polyvinylchloride (PVC) & expanded polystyrene. (EPS). The PUF examined contained PentaBDE and Firemaster 550. ABS contained decabromodiphenylethane. EPS contained HBCD & PVC the plasticizer DEHP. Polymers were crogenically milled & air-jet sieved to generate particles of similar size (53-300 um) for testing. Microplastics were examined by electron microscopy & their surface areas measured. Ground polymer (0.4 g) was mixed with sand and packed into a HPLC column located in a temperature controlled incubator. Water of the desired temperature & humic acid content was pumped through the column at ~1 ml/min, the eluent collected and analyzed by LC or GC/MS. In addition to BFRs, several tris phosphate FRs were detected in the water. These tris phosphates were not detected in the PUF as they were present at relatively low levels. Elevated temperature (40^0C) and humic acid content leached greater amounts of the hydrophobic additives (e.g. DBDPE, PBDEs, TBB). Levels of HBCD in the leachate were surprisingly low.

TP177 Environmental Costs and Benefits of Nano-Silver Textiles: combining LCA and MCDA

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Silver has been utilized for its antimicrobial properties since antiquity. Those same antimicrobial properties have produced a myriad of nano-silver (nAg) enabled products, and in particular nAg textiles. These textiles are currently market as “anti-stink gear”, embracing the notion that the addition of the nAg will inhibit the growth of bacteria on the textile and reduce odor. Manufacturers of these textiles tout the ability to launder them less, and thus reduce the overall life cycle impact of the textile, as laundering has historically been found to be the greatest contributor to the overall life cycle environmental impact of textiles. However, the addition of nAg to textiles comes at an additional environmental cost when compared to conventional textile counterparts. And a wide variability exists with respect to the concentration of silver attached to the textiles, ranging from 0.9 µg nAg per gram of textile to 21,000 µg/g. Losses of nAg due to laundering also vary significantly as a function of the attachment method of the silver, ranging from < 1-100% of the initial silver content of the textile. The loss of silver not only decreases the antimicrobial efficacy of the textile, but also provides a pathway for nAg to enter the environment. The silver will eventually enter the wastewater treatment plant, and is expected to be in the form of AgCl or Ag_2S. Approximately 90-95% of the silver entering the wastewater treatment plant will be removed into the biosolids, with the rest entering the aquatic environment. At the same time there are expected benefits to these textiles, which are not only limited to reduced laundering. In a medical setting, the antimicrobial benefits may vastly outweigh the increased environmental burden of nano-enabling the textile. In order to fully investigate the environmental costs and benefits of nAg enabled textile when compared to the conventional counterpart life cycle assessment is coupled with multi-criteria decision analysis. This case study provides a framework for evaluating the benefits and costs of nano enabled products from an environmental and human health perspective. This methodology has the potential to advance the state of the filed, when assessing the environmental and human health costs and benefits of nano-enabled products.

TP178 Human exposure analysis of airborne halogenated flame-retardants

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Halogenated flame-retardants (FRs) (e.g. PBDEs), used in a wide array of polymer-containing products, are being detected in indoor environments. Positive correlations have been observed between PBDE levels in house dust & those in human breastmilk. PBDEs have been largely phased out, but replaced by other halogenated FRs that lack comprehensive toxicity testing. Positive associations are now being observed between levels of replacement FRs (i.e. 2-ethylhexyl 2, 3, 4, 5-tetrabromobenzoate (TBB) & bis (2-ethylhexyl) 3, 4, 5, 6-tetrabromophthalate (TBPH)) in house dust & on handwipes of children. Inhalation exposure for several chlorinated organophosphate FRs (ClOPFRs: tris (1, 3-dichloro-2-propyl) phosphate (TDCPP), tris (2-chloroethyl) phosphate (TCEP), & tris (1-chloro-2-propyl) phosphate (TCPP)) was estimated to exceed exposure from dust ingestion, indicating that the toxicological importance of inhalation of indoor airborne FRs. However, not all airborne particulates are respirable. Larger particulates can be trapped on the mucosal lining of the respiratory tract, then expelled or swallowed. Therefore, to explore the route of airborne FR exposure, we compared respirable (< 4µm) & inhalable (> 4µm) personal air levels within common indoor spaces (i.e. residences & mixed (office/residences)) to levels detected in gymnasiums. The latter environments are laden with FR-treated polyurethane foam & have previously been reported to contribute to gymnast PBDE blood levels. Within residences, office/residences & gyms, the air particulates > 4µm carried > 93% of the FR burden, consisting mostly of TCPP. Total ∑ClOPFRs levels in personal air showed no significant difference among the three environments ($p > 0.05$), with levels of 595, 428, 546 ng m^{-3} (respirable + inhalable), respectively. Total Penta-PBDEs & TBB + TBPH levels within the gyms were < $1/3^{rd}$ of the ∑ClOPFRs concentrations, but exceeded those in residences & office/residences by several fold ($p < 0.05$). However, the respirable air levels (< 4µm) of TBB + TBPH in all three environments & Penta-PBDEs levels between the gyms & residences were not significantly different ($p > 0.05$). Although the gym air levels exceeded those of the residences & office/residences for some FRs, these findings indicate that the respirable exposure may be similar between these spaces. These findings indicate that ingestion of airborne particulates should be considered in future risk assessments.

TP179 Models for Estimating Exposure to Chemicals used in Consumer Products and Indoor Environments

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The U.S. Environmental Protection Agency’s Office of Pollution Prevention and Toxics (OPPT) assesses exposures, including consumer exposure, for its New and Existing chemicals Programs. Consumer exposure estimates can be based on measured exposure data, modeling approaches, or a combination of both. OPPT’s Consumer Exposure Model (CEM) was recently updated to include six inhalation models, five ingestion models, and four dermal models that can be used to approximate nearly 100 product and article exposure scenarios. All CEM models are used to estimate time-varying chemical concentrations in exposure media, including the product or article itself, indoor air, airborne particles, settled dust, and soil. The model also evaluates dermal flux of a chemical through the skin from contact with liquid or gas-phase chemical, and estimates the migration of a chemical from an article into saliva. OPPT has also developed a higher-tier model based on EPA’s Office of Research and Development’s existing Indoor Air Quality and Inhalation Exposure (IAQX) and Indoor-Semi-Volatile Organic Compound (I-SVOC) models. This new OPPT model is a simulation program for estimating chemical emissions from consumer products, articles, and building materials and their impact on Indoor Environmental Quality in Buildings with Conditioned and Unconditioned zones (IEQ-CU). IEQ-CU characterizes short and long-term emissions of volatile organic compounds (VOCs) and SVOCs and estimates subsequent air, airborne particle, and dust concentrations. CEM was developed for wide application for both data-poor and data-rich chemical and use scenarios. IEQ-CU was developed for refined estimates of exposure and requires more model inputs than CEM. Selection of the most applicable model depends on the scenario under evaluation, the chemical and pathway of interest, and the quantity and quality of available data. This presentation will provide an overview of approaches to measure or estimate required CEM and IEQ-CU model inputs such as emission rates, gas-phase mass transfer coefficient, material-air partition coefficient, and solid-phase diffusion coefficient, source dimensions, inter-zonal air flow, and initial concentrations of chemical in the source. Potential next steps for model development and application will also be discussed. The views expressed in this abstract are those of the authors and do not represent Agency policy or endorsement.

TP180 Testing Protocols for Evaluating Exposure to Chemicals from Products

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The U.S. Environmental Protection Agency's Office of Pollution Prevention and Toxics (OPPT) assesses exposures, including consumer exposures, for its New and Existing Chemical Programs. Consumer exposure estimates are based on available product testing data, monitoring data, or modeling approaches. Existing methods have been used by organizations to test products for chemical content and emissions. There is variation and lack of consensus across testing protocols that evaluate exposure to chemicals from products. Such protocols are typically tailored to the scenario of interest and will vary based on information about chemical use and measured or estimated physical-chemical properties. However, some aspects of product testing could be standardized to promote efficiency, data comparability, and more routine use of such data in exposure assessments. OPPT developed a first set of generic exposure testing protocols in 2015 and is now updating those protocols. The updated protocols have information on experimentally measuring the chemical content in products or articles; emission rates, including short-term emission rates, long term emission rates, and determination of material-air partition coefficients and solid-phase diffusion coefficients; and generation of particles by abrasion. The updated protocols also have information on direct transfer of chemical to dust in contact with the article, chemical transformation due to photolysis, migration/leaching of chemical from article into water, migration of chemical from article into saliva and sweat, and dermal exposure to chemicals transferred from liquids or particles to the skin. This presentation will provide an overview of testing protocols and general considerations for how these testing protocols could be applied to different chemical/product or chemical/article combinations. This presentation will also summarize how results from product testing could be used directly in exposure assessment or as inputs for available consumer exposure models. The views expressed in this abstract are those of the authors and do not represent Agency policy or endorsement.

Science of Sediment Toxicity Testing: Method Advances, Interpreting Results and Use of Data in Ecological Risk Assessments

TP181 Future Challenges in Sediment Toxicity Testing for the Risk Assessment of Plant Protection Products

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Sediment toxicity testing is gaining an increasing awareness within the scientific community. In 2015, a scientific opinion on environmental risk assessment for sediment organisms was published by the European Food Safety Authority (EFSA). In the EU, this scientific opinion is of high interest for risk assessors and aquatic ecotoxicologists because so far, only the Tier I risk assessment for sediment organisms was addressed in the existing aquatic guidance document published in 2013 by EFSA. In addition, the number of currently available standardized and validated OECD guidelines is limited. These tests mainly cover invertebrates (e.g.,Chironomus riparius, Lumbriculus variegatus). A sediment test with the macrophyte Myriophylum is also available. In the United States, sediment toxicity is considered differently within the risk assessment as reflected by the ASTM and USEPA guidelines. There are also a higher number of standardized test methods available for sediment dwelling or epibenthic organisms (e.g.,Hyalella azteca, Chironomus dilutus, Leptocheirus plumulosus). There are a number of differences between the OECD and USEPA guidelines, including the use of natural or artificial sediment, equilibration time, and a flow-through or static test design. These test method differences lead to changes in the physiochemistry of the sediment, in the bioavailability of the test compound, and the concentrations of the test substance in the overlying water, pore-water, and bulk sediment. Due to these differences, the test results of studies performed according to OECD and USEPA test methods are difficult to compare. In recent years, it has been discussed which matrix (overlying water, pore-water, sediment, or total loading) should be used to determine effects endpoints in sediment toxicity studies. Most of the test organisms used are epibenthic and live on the sediment surface, rather than in the sediment itself. Therefore, being aware of the discrepancies between the OECD and USEPA test methods, some common issues need to be considered. These issues include the relevant route of exposure, as well as how to express test results to be used in the environmental risk assessment.

TP182 Interlaboratory Evaluation of Bioaccumulation from Field Collected Sediments Using Hexagenia spp., Lumbriculus variegatus and Pimephales promelas

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Standardized bioaccumulation testing of aquatic organisms is essential to understanding the impact of historical contamination on the quality of water and sediment. Input of contaminants into aquatic ecosystems has been reduced through the regulation of discharges, however historical contamination continues to impact the quality of water and sediment. In addition to the physicochemical characterization of sediment, toxicity testing, and benthic surveys, an assessment of the bioaccumulation of contaminants is now part of the decision-making framework for contaminated sediments in Ontario and elsewhere. A standardized freshwater sediment bioaccumulation method for the mayfly Hexagenia spp., the oligochaete Lumbriculus variegatus, and the fathead minnow Pimephales promelas, has been developed and validated by the Ontario Ministry of the Environment and Climate Change (MOECC). Interlaboratory testing is needed to understand precision and variability of bioaccumulation test results and assist with the interpretation of bioaccumulation data. An interlaboratory comparison was conducted to assess the precision of this method. Three field collected non-toxic sediments contaminated with bioaccumulative compounds were used in this study. Sediment contaminated with arsenic was tested with Hexagenia spp., polycyclic aromatic hydrocarbon (PAH) contaminated sediment was assessed using L. variegatus, and sediment with high levels of polychlorinated biphenyls (PCBs) was tested with P. promelas. Control and test sediment were sub-sampled and sent to six (Hexagenia spp.) or seven (Lumbriculus variegatus and Pimephales promelas) laboratories to perform the bioaccumulation test. Survival, growth and tissue accumulation of the contaminant of concern will be presented and method precision, challenges and opportunities for method refinement will be discussed.

TP184 An evaluation of endpoints from benthic invertebrate chronic toxicity tests based on draft US FIFRA guidance – Part II

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On October 26, 2007, sediment chronic toxicity testing with benthic aquatic invertebrates became a conditional requirement as part of the Office of Pesticide Program's ecological effects data requirement contained in 40 CFR Part 158 Subpart G. Studies are now required for any pesticides with soil partition coefficient (Kd) ≥ 50, log Kow ≥ 3, or Koc ≥ 1000. Due to the novelty and complexity of the study designs, it is important to gain a critical understanding of the relative sensitivities of required test endpoints within and among tests. At the 2015 SETAC North America annual meeting, the CropLife America (CLA) Ecotoxicology Work Group Sediment team presented a poster detailing key findings from a sediment toxicity testing database that was compiled by CLA. For the Chironomus dilutus life-cycle tests, larval survival, growth and emergence were the most sensitive parameters, whereas endpoints based on reproduction or adult survival (i.e., number of days to death) were consistently less sensitive. For, for chronic sediment studies with amiphipods (Hyalella azteca and Leptocheirus plumulosus), survival, growth, and reproduction proved to be important parameters to measure and each parameter yielded the most sensitive endpoint for certain studies. This presentation will provide an update and recommendations for streamlining relevant endpoint selection, as well as more closely consideration of the variability within control responses and subsequent implications.

TP185 Development of whole-sediment toxicity identification evaluation (TIE) techniques for use in Australia

W.T. Mehler, Univ of Melbourne / Zoology; V.J. Pettigrove, The Univ of Melbourne / Zoology

A majority of the public literature and available guidance documents on "how-to" conduct whole-sediment toxicity identification evaluations (TIEs) detail the use of test organisms and amending agents that are readily available in the United States. Although much of the information in these documents can be utilized regardless of location, the test organisms and amending agents documented in the current TIE literature are both not available and largely inappropriate (i.e. not native species) for conducting whole-sediment TIEs in Australia. Thus, the overall objective of this study was to build foundational methodologies for performing whole-sediment TIEs with native Australian species. In this study, we examined the capability of three readily available amending agents in Australia: a local produced zeolite product (for ammonia), an activated carbon (for non-polar organics), and Lewatit Monoplus TP 207 (for cationic metals) on two Australian native freshwater species: Chironomus tepperi (midge) and Austrochiltonia subtenuis (amphipod). To evaluate the effectiveness of each amendment, bioassays were conducted with spiked sediments of ammonia, permethrin, and copper using mortality and growth as endpoints. For each amendment, the effectiveness in reducing toxicity differed based on which test organism was used. Understanding the differences in testing methodologies (such as test duration), species sensitivities, and the niche and behavior of the organism is impetrative for building a solid foundation for TIE work in areas were these techniques have yet to be applied.

TP186 Defining Equilibration Time of Spiked Sediments for Use in Whole Sediment Toxicity Testing: A Post Hoc Assessment

M.J. Bradley, Smithers Viscient / Sediment Toxicology

Spiked whole sediment toxicity testing in support of US pesticide registration typically utilizes a prolonged aging period of spiked sediments prior to organism exposure, during which concentrations of the applied test compound presumably achieve a state of equilibrium between sediment and pore (interstitial) water. In recent years, regulators have requested that compound-specific equilibration periods be defined prior to organism exposure, as opposed to using default aging periods (e.g., 30-days) described in older test methods. A test design for determining a compound-specific equilibration period can be conceptualized relatively easily, however, interpretation of the results can be made difficult by multiple confounding factors, most notably the adsorptive qualities of the compound, organic carbon content of the sediment-pore water matrices, and the conventional analytical methods employed (e.g., liquid-liquid extraction). While partitioning of a compound with a relatively low adsorption coefficient can be interpreted rather clearly, compounds with a higher adsorption coefficient may result in more variable data, which also may not be considered realistic or relevant to anticipated aqueous concentrations (e.g., measured values exceeding solubility). These findings are generally attributable to the suspended and dissolved organic carbon content of a pore water sample to which the compound may be bound, which ultimately leads to an over estimation of freely dissolved compound following extraction and analysis. The uncertainty of these data results in a subjective interpretation which may thereafter be contended or be considered inconclusive. This presentation will review a number of data sets related to compound-specific sediment-pore water equilibration studies to look at the relevance and reliability of the data collected, in light of varying compound physicochemical attributes and assumptions of equilibrium partitioning theory. Ultimately, the goal of this presentation is to refine an approach for objectively determining an appropriate compound-specific equilibration period for spiked sediment testing, while understanding the limitations of the methods employed.

TP187 Development of site-specific sediment quality guidelines for a tropical region (Ceará State, Northeast Brazil)

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Dredging operations involves the excavation and removal of sediments and rocks from the bottom of harbors zones for the maintenance of depth of navigation channels. However, sediment contamination can cause environmental impacts and economic losses, offering ecological risks to animals exposed to such material, both at the time of dredging as in the oceanic disposal. To deal with this environmental concern, normative enforcements and criteria have been created to support management actions. Such criteria involve the development of sediment quality guidelines (SQGs) through different approaches (e.g. theoretical, empirically and site-specific) which represent thresholds that reflect the relationship between concentrations of contaminants and adverse biological effects. In Brazil there are differences in climate and sedimentology along the coastal zone resulting in different conditions of geochemical aspects that control the bioavailability of contaminants in sediments that need to be addressed. Also, The Federal Resolution CONAMA 454/12 was published with the purpose of establishing SQGs for dredging activities in Brazil, but however, such parameter were based on international values and fails to predict toxicity, supporting the need of site-specific assessments. In this study we developed site-specific SQVs for harbor areas of Ceará State located in the Tropical region of Brazil, based on an integrated analysis of sediment contamination (trace metals and hydrocarbons) and sediment toxicity of solid and liquid phases by using multivariate analysis and geochemistry indices calculated from background values. The results allowed the identification of chemicals of concern and the toxic effects range related to sediment contamination. Also, site-specific SQGs are more restricted than SQGs applied in most countries, including Brazil supporting the hypothesis that the aplication of international SQGs may result in failures to predict impacts associated with dredged materials.

TP188 The Importance of Sediment in Aquatic Toxicology: Refined Hazard Assessment of the POEA surfactant in Roundup

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Due to the lack of exposure data, it has been challenging to assess the potential ecological hazard/risk posed by MON 0818, the polyoxyethylene amine (POEA) surfactant present in the original formulations of glyphosate (e.g. Roundup Original). To date, hazard/risk assessments for this surfactant have been performed following traditional Tier-1 approaches. These are based on acute (24-48h), water-only toxicity tests compared to worst-case-scenario water-column concentrations assuming constant exposure over the duration of the experiment. We recently showed that MON 0818 presents a high affinity for sediment and that, in its presence, the water column half-life of the surfactant can be very short (generally < 10h, and commonly < 5h). Here, we present a refined hazard assessment of MON 0818 based on the re-evaluation of the existing toxicity data, supplemented by water-only toxicity data for an additional 15 species, tested with standard methodologies and confirmation of exposure concentrations. In order to further explore the role that sediment plays in the exposure of aquatic organisms to MON 0818, we also present the results of a series of test following novel approaches such as water column toxicity tests in the presence of sediment and the evaluation of pulse-recovery approaches to mimic water column exposures for compounds with short water-column half-life. When evaluated in this manner, no acute (24-h) mortality was observed for three of the five species tested in the presence of clean sediment up to 10 mg L^{-1} MON 0818 initial water concentration. The EC50s for the other two species were significantly greater than for parallel water-only tests. Our results indicate that quick dissipation of MON 0818 in the presence of sediment can reduce the effects on exposed organisms, and that full recovery from 24-h exposures to concentrations of MON 0818 equal to, or greater than, those expected in the environment is possible. These results highlight the inappropriateness of simple screening-level approaches for the assessment of the risk posed by compounds for which the expected exposure regimes may deviate from that more typically used in traditional standard acute laboratory tests. For compounds with similar characteristics, toxicity testing in the presence of sediment can serve as a valuable tool, even when the sediment is not the initial source of exposure.

TP189 Toxicity of Pharmaceuticals and Personal Care Products to the Estuarine Amphipod, Leptocheirus plumulosus, Exposed to Sediments from an Urban Waterway

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For sediments in industrial urban waterways, benthic invertebrate toxicity is often used as a basis for developing preliminary remediation goals for chemicals such as polycyclic aromatic hydrocarbons (PAHs) and metals. Understanding how both legacy and ongoing sources (from run off and combined sewer overflows) of contamination contribute to ecological risk are important for informing such sediment remedial decisions. Furthermore, pharmaceuticals and personal care products (collectively referred to as PPCPs) represent a group of chemicals that are commonly found in sediments from urban waterways, but are often not analyzed for or considered in ecological risk assessments. Sediment chemistry and 28-day benthic toxicity data (using the estuarine amphipod, Leptocheirus plumulosus) were evaluated from Newtown Creek, a large industrial intertidal waterway in New York, which receives significant input from CSOs and stormwater run off. The objective of this study was to evaluate whether there were any apparent relationships between PPCP sediment concentrations and benthic toxicity. Comparisons were also made to more typical ecological risk drivers, such as PAHs and metals. Dose-response curves were generated using toxicity data compared to both bulk sediment concentrations and estimated pore water toxic units (TU). Several of the PPCPs analyzed, namely bisphenol A, nonylphenol, and 4-tert-octylphenol, showed clear concentration-dependent relationships to benthic toxicity. These results suggest PPCPs could be contributing to the overall toxicity observed in benthic invertebrates exposed to Newtown Creek sediment. The sum of the estimated pore water TU for these three PPCPs also showed a strong relationship to benthic toxicity, particularly survival. Statistical analysis further indicates PPCP TUs are strongly correlated with toxicity (Pearson's correlation coefficient = -0.80). Additional studies would be needed to confirm these results given the uncertainty of the pore water PPCP TUs, which were based on pore water concentrations estimated from equilibrium partitioning.

TP190 Recent advancements in sediment elutriate (dredged material) water column evaluations

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Evaluations to determine the suitability of dredged material for open water placement may require physical, chemical and biological evaluation of the sediment in the suspended phase and solid phase. Bioassays are used to determine potential acute toxicological effects to pelagic organisms, and acute and chronic implications to benthic organisms. This work focuses on evaluations of the suspended particulate and dissolved chemical phases in the water column while dredged material settles in open water. While greater concern may be on the acute effects of persistent contaminants such as metals, ammonia often causes toxic effects in elutriate toxicity bioassays. In this presentation, freshwater (Ceriodaphnia dubia, Pimephales promelas) and marine (Americamysis bahia, Menidia beryllina, Mytilus edulis, Arbacia punctulata, Arcatia tonsa, Pseudodiaptomus pelagicus) organisms were exposed to both suspended sediments (elutriates) and model contaminants (copper, ammonia) that may be released to the water from suspended sediments. Comparison between marine test species indicated dramatic differences in sensitivity, most notably to ammonia. Recently adapted ammonia toxicity reduction evaluation methods for elutriates (zeolite, pH reduction, EDTA, C18) were employed to determine that ammonia was the main driver of toxicity in specific freshwater and marine elutriates (survival increased from < 20% to >90%), and metals and organics were not at sufficient concentration to induce mortality. These data were used develop support for applying alternative safety (application) factors for ammonia, rather than the default 0.01 factor, loosely based on a NOEC/LC50 ratio. Low effect endpoints (EC5, EC10) were modeled due to criticism of the NOEC. In addition, testing with Mytilus embryo development endpoints indicated extreme sensitivity to unionized ammonia (EC50 = 0.17 mg/L) questions need for a safety factor embryo development is the organisms' most sensitive life stage, based on chronically exposed juvenile mussels being two times more tolerant. Since urchin and mussel embryos are not holozooplankton, international marine copepod methods were adapted and indicated that while copepods (Acartia, Pseudodiaptomus) are very sensitive to copper, they are less confounded by ammonia. These data support methods to make relevant and representative risk-informed decisions for dredging evaluations.

TP191 Exposure to Sediments Spiked with Deepwater Horizon Oil Inhibited Growth in Juvenile Red Drum and Pacific White Shrimp

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In support of the Deepwater Horizon (DWH) Natural Resource Damage Assessment, we exposed juvenile (21 days post-fertilization) red drum (Sciaenops ocellatus) and post-larval Pacific white shrimp (Litopenaeus vannamei) to sediment spiked with DWH oil. We initiated these tests simultaneously using identical sediment spiked with naturally weathered DWH oil and identical flow-through exposure systems. We exposed juvenile red drum for 13 days and Pacific white shrimp for 6 days. Exposure to DWH oil in sediment inhibited growth in both species. The 13-d EC20 for red drum growth was 37 mg/kg TPAH50 (sum of 50 polycyclic aromatic hydrocarbons). The 6-day EC20 for Pacific white shrimp growth was 3.9 mg/kg TPAH50. We will discuss the methods used to conduct these bioassays, present our results and compare them to growth- inhibition effects we observed in similar bioassays that we conducted with amphipods (Leptocheirus plumulosus) and southern flounder (Paralichthys lethostigma) using sediment spiked with DWH oil. We will also discuss all of these results in the context of sediment equilibrium partitioning theory with PAHs.

TP192 Human health and ecological risk assessment of a Department of National Defense water lot

I. Koch, Royal Military College of Canada / Environmental Sciences Group; R. Lavoie, Royal Military College of Canada / Biology; T. Laing, V. Paquin, D. Loock, K.P. Weber, Royal Military College of Canada / Environmental Sciences Group

A human health risk assessment (HHRA) and an ecological risk assessment (ERA) were performed at a Department of National Defense water lot. Previous and concurrent site assessments indicated the presence of inorganic elements, polychlorinated biphenyls (PCBs), petroleum hydrocarbons (PHCs), tributyltin (TBT), and polycyclic aromatic hydrocarbons (PAHs) in sediment at concentrations that exceeded guidelines. The overall objective of the study was to determine whether there are potential human health and ecological risks associated with contaminants in sediments at the site to help inform risk management and remediation options. Water, sediments, and fish were analyzed for contaminants. Benthic comparative and toxicity evaluations were completed. Risks to macrophytes, fish, mammals, and birds were evaluated using uptake models and food chain modelling. For the HHRA, the chemicals identified as contaminants of potential concern in sediments through the screening process were arsenic (As), chromium (Cr), lead (Pb), PCBs, and PAHs. No guidelines were exceeded for water. Concentrations of chemicals in fish tissues were compared with tissue guidelines and from reference locations; PCBs as well as PAHs represented a risk. Unacceptable health risks were predicted for the exposure scenarios for human receptors. Risks were predicted from PCBs via fish consumption and from As, Pb, PCBs, and PAHs via sediment contact. For the ERA, the chemicals identified as contaminants of potential concern in sediments were As, barium (Ba), copper (Cu), Pb, mercury (Hg), tin (Sn), zinc, (Zn), TBT, PAHs, PHCs, and PCBs. Risk was assessed for benthic organisms by comparing the benthic community structure at the water lot with reference site. Significant effects on taxonomic richness, diversity, and proportion of tolerant taxa were also seen at the water lot compared to the reference site. The risk to the benthic community was also assessed by evaluating the survival and growth of Hyalella azteca and Chironomus dilutus test organisms exposed to site sediments in laboratory chronic toxicity tests. Significant effects were seen for growth rate and survival of test organisms at the water lot compared to the reference site. Risk was predicted for wildlife from inorganic elements, PAHs, PHCs, and PCBs. This presentation will highlight some of the challenges in performing HHERAs and how results can differ from federal guidelines in the context of making contaminated site management decisions.

TP193 Transcriptional profiling of a native estuarine amphipod exposed to road dust and the related chemicals by cDNA-AFLP

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Benthic environment is usually contaminated with mixtures of chemicals and can cause adverse effects on ecosystems. Recently transcriptomic analysis has been demonstrated to have the potential to identify causative toxicants in contaminants, which can aid environmental decision making. However studies on transcriptional analysis using a native species are still lacking, since most of the studies have used model species whose sequences were well-known. This study aimed to investigate the potential of transcriptional analysis using a native species to link the biological effects and the causative stressors. We performed transcriptional profiling of a native estuarine amphipod Grandidierella japonica exposed to sublethal levels of road-deposited dust and the contained substances such as Cu, Zn and nicotine for 24 h by cDNA-amplified fragment length polymorphism (AFLP) analysis with 12 combinations of arbitrarily selected primers. The estuarine amphipods were collected from a tidal flat near Tokyo, and the road-deposited dust was collected from highways around Tokyo, Japan. Our results demonstrated that the most of transcripts-derived fragments (TDFs) were differentially expressed in response to only one chemical, and that about 10% to 30% of TDFs were up- or down-regulated in common between different chemicals. These specific TDFs might be the possible biomarkers to detect each chemical exposure. However there were also few up- or down-regulated TDFs in common between the road dust sample and reference chemicals, which implied the contribution of unmeasured toxicants to gene expression profile of road dust or/and the interaction of measured toxicants. These results were consistent with the previous study in which the measured toxicants (Cu, Zn and nicotine) could not explain the observed road dust toxicity. Although the causative toxicants have not been identified yet, the current study showed the specific transcriptional patterns to chemical exposure and provides the experimental support for the potential utility of transcriptional analysis using a native species.

TP194 Direct and indirect toxicity of single walled carbon nanotube to freshwater organisms

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Production and development of carbon nanomaterials has increased for the past few years. In Canada, the forestry industry experienced an economic crisis which has led to the elaboration of new forestry products: nanomaterials, including single-walled carbon nanotubes (SWCNT). Because carbon nanomaterials have a large specific surface area giving them high adsorption capacity, they are closely studied for drug targeting and water treatment. Indeed, this property is very interesting for the removal of contaminants like metals in wastewater. Adsorption of metals is greater for purified or functionalized carbon nanotubes. Production increase of nanomaterials and applications for water treatment raises the question of their direct but also indirect toxic effects on aquatic organisms which may be exposed. The adsorption properties of SWCNT may alter the bioavailability of contaminants present in the medium and change the global toxicity of the mixture.

Issues, Challenges and Opportunities for Protection of Water Resources from Emerging Organic Contaminants

TP195 Combining bacterial loading and nutrient loading analyses to point source agricultural impacts from large livestock facilities

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Large livestock facilities called Confined Animal Feeding Operations (CAFOs) have been the source of significant nutrient and bacterial loading in the nation's lakes and streams. Hypoxic zones in Chesapeake Bay, Lake Erie and the Gulf of Mexico have been attributed to CAFO impacts. In Michigan, there are over 230 registered CAFOs which threaten the health of the Great Lakes and their associated watersheds, as well as the health of the people who live in them. Gratiot County Michigan has one of the highest concentrations of CAFOs (23) in the state and has measured significant impacts all over the local drainage basin. These impacts include high concentrations of nutrients and coliform bacteria (exceeding state and EPA standards) as well as extremely low concentrations of dissolved oxygen producing hypoxic and anoxic conditions. Additionally, significant antibiotic resistance has been found in E. coli associated with CAFO impacts within the watershed. Problems with changing current policy and regulatory structures are hampered, in part because of non-point source designation of this kind of aquatic pollution. Past research has utilized statistical relationships between nitrogen ammonia (NH_3) and soluble reactive phosphorus (SRP) to identify CAFO facility inputs and manure application sites as point sources. If inputs from animal waste impact waterways within a relatively short time, a strong correlation between NH_3 and SRP will occur. Calculation of potential septic system inputs utilizing the NH_3 / SRP correlation, however, is more problematic since nutrients can take a relatively long time from septic system to nearby waterway and thereby allow NH_3 volatilization and SRP uptake by plants. This study employed the method of combining: 1. NH_3/SRP correlations and E. coli concentrations, and 2. NH_3/SRP correlations and fecal coliform fingerprinting to enhance the effectiveness of point sourcing nutrient and bacterial inputs from both CAFOs and septic systems. If these methods prove effective, it will provide a reliable means by which inputs can be identified and designated as point source. That would change the regulatory landscape and allow environmentally-friendly restrictions to occur more easily.

TP196 Fate and Mass Balance of Pharmaceuticals, Personal Care Products, and Hormones in Septic Systems of Drainfields

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Household wastewater discharged from septic systems, a common type of onsite wastewater treatment systems (OWTS), can be a potential source of micropollutants in the environment. This study investigated the fate and mass balance of 17 micropollutants (i.e., wastewater markers, pharmaceuticals, personal care products, and hormones) in the drainfields of OWTS. Three replicate drainfields (1.5 m length, 0.9 m width, and 0.9 m height) were constructed and managed similar to the field practice. Each drainfield received 9 L of septic tank effluent (STE) per day (equivalent to 32.29 L/m_2 per day) that dispersed from a drip line with over 8 months. We detected 17 micropollutans in STE and 12 in the leachate from OWTS at variable concentrations. Three micropolutants, including acetaminophen, carbamazepine, and sulfamethoxazole were observed from the soil of OWTS at the end of the study. Approximately 85% of effluent-borne micropollutants attenuated withinin 60 cm deep of OWTS, suggesting that vadose zone processes such as sorption and microbical degradation likely limited transport of micropollutants from the OWTS. Further, maintaining aerobic conditions in parts of the OWTS are needed to effectively remove micropollutants. We suggest that a better understanding of micropollutnts in OWTS is needed to protect groundwater quality.

TP197 Incidence of veterinary pharmaceuticals in West Texas playa wetlands

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Veterinary pharmaceuticals, such as antibiotics, steroid hormones, and β-adrenergic agonists, are commonly used to promote weight gain and increase feed efficiency at beef cattle feedyards. These veterinary pharmaceuticals have been found in surface water and groundwater downstream of cattle feedyards. Veterinary pharmaceuticals can also be transported via airborne particulate matter. However, it is unknown whether wind-blown dust is a substantial transport mechanism for veterinary pharmaceuticals entering adjacent surface waters. This study assessed the incidence of veterinary pharmaceuticals in playa wetlands of the Southern High Plains region of West Texas. Playa wetlands located approximately 1-10 miles from a feedyard were included in this study due to potential for entrapment of feedyard dust but not wastewater. Water and sediment samples were obtained from 32 individual playa wetlands and analyzed using LC-MS/MS to quantify veterinary pharmaceuticals indicative of feedyards: 5 antibiotics, 6 steroid hormones, and a β_2-adrenergic agonist (ractopamine). Preliminary results indicate that 61% of sampled playa wetlands contain veterinary pharmaceuticals. These chemicals have potential to disrupt endocrine function and alter microbial community composition and dynamics. Results of this study have implications for aquatic wildlife reproduction and potentially human health in arid or semi-arid regions that contain beef cattle feedyards.

TP198 Reusing Wastewater in Agroecosystems: Groundwater quality, plant uptake, and antibiotic resistance?

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With demands on water supplies rising, wastewater treatment plant (WWTP) effluent is often reused to irrigate agricultural lands. Emerging contaminants, like antibiotics, are frequently found in effluent due to limited removal during WWTP processes. Concern has arisen about their environmental fate, particularly possible contamination of groundwater, plant uptake, and health effects linked to antibiotic resistance in the environment. The aim of this study was to examine the environmental impacts of spray irrigating WWTP effluent on cropped lands at The Living Filter, a wastewater reuse site, by quantifying three antibiotics: sulfamethoxazole (SMX), trimethoprim (TMP), and ofloxacin (OFL), in WWTP effluent, soil, groundwater, and wheat plants (Triticum aestivum) and providing analysis of antibiotic resistance in environmental bacteria. Water samples were collected three times annually, while wheat was collected once and soil was collected twice in the summer. Throughout the year, the three antibiotics are present in WWTP effluent and groundwater. Effluent concentrations range from 2 ng/L to 22 ug/L and vary based on the time of the year. In groundwater, SMX and OFL are quantifiable with a concentration range of 0.37 – 313 ng/L, and TMP is typically only detectable, but had a high concentration of 22 ng/L. Considering the concentrations associated with wheat plants, OFL was found in straw (10.2 ± 7.05 ng/g) and grain (2.28 ± 0.89 ng/g); TMP was only found on wheat plant surfaces; and SMZ was concentrated within the grain (0.64 ± 0.37 ng/g). Concentrations in soil by depth will also be presented. Based on data to date for antibiotic resistance in soil bacteria, antibiotic resistance to OFL was absent, while resistance to SMX and TMP was elevated compared to a control site not receiving WWTP effluent nor manure applications. These findings indicate that antibiotics are present in groundwater and wheat plants following irrigation with WWTP effluent. Additionally, the introduction of human antibiotics appears to impact antibiotic resistance in soil bacteria. Overall, this study suggests that antibiotics introduced into the environment due to WWTP reuse may pose potential risks to the long-term health of ecosystems, with consequences for human and animal health due to antibiotic resistance. However, additional research is necessary to determine the full environmental and health implications of reusing WWTP effluent for the purpose of irrigating agricultural lands.

TP199 Concentrations of bisphenol A in North American surface waters: calculating from summary statistics used in deterministic and distributional risk analyses

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Bisphenol A (BPA) is mainly used as a chemical intermediate to produce polycarbonate plastics and epoxy resins. During and following manufacturing, processing, use, and disposal of polycarbonate and epoxy resin products, as well as other products containing BPA as a functional additive, some BPA can enter wastewater treatment plants for treatment or landfills for disposal, and thus may reach surface waters. BPA is frequently included as an analyte in surface water sampling programs targeting compounds of emerging concern. Concentrations of BPA in North American fresh and marine waters were compiled from published scientific literature and governmental reports. The dataset now contains ca. 2300 entries of data from 85 sources. Three issues were identified that needed to be addressed to enable calculation of summary statistics. First, most of the measured values were actually below a detection limit and detection limits varied over several orders of magnitude across all studies. The Kaplan-Meier method was used to estimate concentrations below detection limits from the distribution of values above these detection limits in studies with a lower detection limit. Second, many studies only reported summary statistics such as means or ranges. When the number of samples was known, values were imputed for that number of samples so that the summary statistics of the imputed concentrations were identical to the summary statistics reported and the distribution of the imputed concentrations approximated a log-normal distribution. Third, many sampling campaigns collected samples over broad areas, while others collected multiple samples at few locations thus requiring the use of weighting factors such that each location carried the same weight in calculating summary statistics. For North America, a total of 804 freshwater and 32 marine water weighted observations are available. For freshwater locations, 72% were below a detection limit (varying detection limits). Median and upper 95th centile concentrations for the full distribution of weighted observations were 0.005 and 0.30 µg/L. For marine locations, 49% were below a detection limit. Median and upper 95th centile concentrations were 0.0011 and 0.023 µg/L. The distribution of weighted observations of BPA will be compared to the distribution of aquatic ecotoxicity data for freshwater and marine organisms to estimate potential risks.

PFAS: Recent Developments in Science, Risk Assessment and Regulation

TP200 Avian Exposures to Seven Perfluorinated Alkyl Substances in Aquatic Habitats Impacted by Aqueous Film Forming Foam Releases

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Information on the adverse effects of perfluorinated and polyfluorinated alkyl substances (PFAS) in animals is primarily limited to perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS). While detectable levels of PFOS and PFOA in environmental media are known to be present at many sites as a result of Aqueous Film Forming Foam (AFFF) releases, less is known about other prominent PFAS chemicals. Since birds may have relatively high exposure potential due to consumption of fish, invertebrates, and sediment in aquatic habitats affected by AFFF releases, avian exposures were evaluated for seven PFAS chemicals (PFOS, PFOA, perfluorodecanoic acid (PFDA), perfluorononanoic acid (PFNA), perfluorodecane sulfonate (PFDS), perfluorohexanoic acid (PFHxA), and perfluorohexane sulfonate (PFHxS)) using measurements from four AFFF sites. Exposure modeling was conducted for four avian receptors representing various avian feeding guilds including: lesser scaup (Aythya affinis) and spotted sandpiper (Actitis macularia), Great blue heron (Ardea herodias), and osprey (Pandion haliaetus). For all measured PFASs at all four sites, predicted exposures to the spotted sandpiper were the highest of the four species (excluding one instance where exposure to PFDA for the osprey was higher), by a factor ranging from three-fold to four-thousand fold. At the 4 sites, 70 to 98% of the modeled daily total PFAS exposures for the spotted sandpiper, lesser scaup, and great blue heron were comprised of PFOS while 2 to 11% were comprised of PFHxS, < 1 to 8% of PFNA, and < 1 to 7% of PFDA. For osprey, PFOS exposure accounted for 83-99% of the modeled daily total PFAS exposure followed by 1-6% PFHxA, < 1-4% PFNA, and < 1-4% PFDS. These results suggest the need for ecological modeling at sites potentially affected by AFFF releases to include multiple representative species and expand the current focus on PFOS and PFOA to other bioaccumulative PFAS chemicals. This evaluation enhances risk management and risk communication by identifying additional PFAS chemicals (e.g., PFHxS, PFHxA, PFNA) that have the potential to influence ecological risk-based decision making at AFFF sites.

TP201 Do we understand ongoing sources of perfluorooctane sulfonic acid and its precursors? Lessons learned from developing a new global emissions inventory

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Among the family of per- and polyfluoroalkyl substances (PFASs), perfluorooctane sulfonic acid (PFOS) and its precursors may have attracted the most attention. Between the late 1950s and early 2000s, they were favored by various industry sectors and widely used in numerous commercial and consumer applications for their unique characteristics such as high surfactant ability and durability/persistence. After their worldwide presence was revealed and 3M's voluntary global phase-out in the early 2000s began, they soon drew significant attention from the global regulatory and scientific community. The Scifinder® Database shows that over 3000 peer-reviewed articles in relation to PFOS were published between 2002 and early 2016. Building on the increasing scientific evidence, regulatory and voluntary programs have been initiated with the intention to reduce associated risks. In particular, PFOS and its precursors were added to Annex B (restriction of production and use) of the Stockholm Convention on Persistent Organic Pollutants in 2009. This led to another wave of data being generated on these substances, in particular in developing and transition countries. In light of this increasingly available knowledge and data, we recently developed a new global emissions inventory of PFOS and its precursors. During the course of this project, we identified that despite the vast number of studies available, substantial critical knowledge and data gaps still exist in terms of the production, use, and release of PFOS and its precursors. For example, there is a general lack of adequate information on the use and disposal phase of PFOS and its precursors. Large uncertainties also remain regarding the abiotic and biotic degradation of PFOS precursors including side-chain fluorinated polymers. Here we present these critical knowledge and data gaps, possible implications based on conceptual calculations, and our recommendations to address these gaps. Important to note is that addressing them may not only improve the understanding of the ongoing sources, transport and fate of PFOS and its precursors, but also provide useful insights into understanding the same for other PFASs including fluorotelomer-based substances.

TP202 Evaluation of Site-specific Human Health Risks and Calculation of Risk-based Threshold Concentrations of PFOS and PFOA in Fish

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The United States Environmental Protection Agency recently finalized toxicity reference doses (RfD) and drinking water quality criteria for the perfluoroalkyl and polyfluoroalkyl substances (PFASs) perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA). Along with drinking water and indoor dust, diet items including fish and shellfish may be a significant contribution to human intake of PFASs. To evaluate the potential risks to human health from consumption of fish tissue by recreational fishermen, a Fish Tissue Criteria (FTC) was derived from the RfD for the sum of the PFOS and PFOA. A Relative Source Contribution (RSC) of 20% and 100% were both assumed for evaluation of human health risks from sites with historical PFAS contamination. The FTC for the sum of PFOS and PFOA ranged from 15 ng/g to 73 ng/g, wet weight for a 20% and 100% RSC, respectively. The FTC was compared to empirical concentrations of PFASs in fish from four sites with historic PFAS contamination, and concentrations in fish exceeded the FTCs at 75% of sites using an RSC of 100% and all four sites when using an RSC of 20%. PFOS represented 99% of the sum of the PFOS and PFOA exposure in fish where measured concentrations of both were available. Additionally, a food web model was used to calculate risk-based concentrations of PFOA and PFOS in sediment and water that would be associated with the FTC. The model showed good agreement between modeled and empirical concentrations in fish (when available for a site) with a mean model bias of 0.9 for PFOS and 2.9 for PFOA. Assuming a 20% RSC, absence of model-predicted risk is expected if concentrations of PFOS in sediment and surface water are both less than 0.75 ng/g, dw (assuming 1% OC) and 12.4 ng/L respectively. Assuming a 100% RSC, absence of model-predicted risk is expected if concentrations of PFOS in sediment and surface water are both less than 3.7 ng/g, dw and 62 ng/L, respectively.

TP203 Per- and polyfluoroalkyl substances: Environmental concerns and regulatory measures in the EU

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The European Chemicals Regulation REACH aims at enhancing innovation and competitiveness of the EU chemicals industry and at improving the protection of human health and the environment. Authorization and restriction of the use of chemicals are risk management options under REACH. Per- and poylfluoroalkyl substances (PFASs) are one group of substances partly addressed by these risk management measures. The aim of this presentation is to show which concerns of PFASs lead to risk management measures in Europe, and to introduce these regulatory activities. PFASs are characterised by special properties, e.g. chemical and thermal stability, these substances are used in several industrial as well as consumer applications. They are used as surfactants and polymers in for example textiles, papers, fire fighting foams and biocides. During production of PFASs itself, during their application in different processes and articles as well as during use and disposal of such articles PFASs are released into the environment. A number of long chain perfluoroalkyl carboxylic acids (PFCAs) are listed as Substances of Very High Concern (SVHC) on the REACH Candidate List. These long-chain PFCAs are ubiquitously found in the environment, e.g. in surface water and biota, as well as in humans. To minimize exposure of humans and the environment with C_8 PFCA (PFOA perfluroinated octanolic acid) a restriction of manufacturing, use and placing on the market of C_8 PFCA itself, its salts as well as substances which might degrade to C_8 PFCA has been submitted by Germany and Norway in 2014 and is now on hand of the EU Commission. Moreover the EU-Commission proposed PFOA as a Persistent Organic Pollutant (POP) under the Stockholm Convention in 2015. At the moment a draft risk profile is being developed. Due to regulatory and voluntarily measures the producing companies are switching to short-chain PFASs. Also short-chain PFCAs are persistent. Furthermore they have a high mobility in the aqueous environment, which poses a threat for drinking water. Drinking water contamination with long-chain PFCAs already caused substantial remediation costs. Moreover, there are studies available indicating endocrine disrupting properties of the short chain PFAS. It hase been shown that short chain PFAS can be enriched in plants which could be a posiibility for human exposure as well. To contribute to a sustainable chemistry use of stable perfluoralky moieties should be avoided.

TP204 Summary of an ICPPR Non-Apis Workshop – Subgroup Higher Tier (Bumble bees and Solitary bees) with recommendations for a semi-field experimental design

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The workshop was held from 29 Feb. – 01 Mar. 2016 at the Julius-Kühn Institute (JKI), Braunschweig, Germany. 47 participants took part representing Industry, CRO, authorities and academia. One part of the workshop discussed laboratory testing with bumble bees and solitary bees. The part discussing semi-field design will be presented here. At the semi-field workshop participants presented their experiences in semi-field and field studies. Seven laboratories conducted semi-field or field tests. The majority of tests were done with bumble bees (10 semi-field and 6 field tests). Solitary bee tests were done with the red mason bee (Osmia bicornis) in 6 semi-field and 2 field tests. In general, as no guidance on the design or endpoints was available all tests followed different designs / set-ups. Furthermore, several studies were designed to answer a specific question in a plant protection product registration. Based on the limited experience and the still ongoing discussion about exposure in field studies it was decided to proceed with a semi-field study design. Semi-field in this context relates to tunnel studies with bumble bees and solitary bees. For bumble bees a colony is the smallest experimental unit, for solitary bees the individual bee. For bumble bees it was agreed the main endpoint of a study is queen reproduction. Additional endpoints like flight activity (in crop and entrances) and sugar consumption were not included in the minimum requirements of the future ring test. Solitary bee studies in Europe are at the moment run with Osmia bicornis. This species is commonly called the red mason bee and is used in the laboratory experiments, too. The main endpoint of a semi-field study with O. bicornis will be the number of offspring produced. Assessment of flight/foraging activity is needed as additional information on actively nesting females and on exposure during application. For both organisms it was agreed to establish an experimental test design and run a ring test with interested parties in 2016. The data collected in the ring test will be used in a general database as a basis for further discussions. In the talk the single endpoints will be discussed in more detail and practical examples used to explain the methodology. The semi-field test design recommendation for both species will also be presented in more detail.

TP205 Wildlife TRVs for Emerging Chemicals: PFOS, PFOA and PFBS

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This presentation provides a practical case study applying the overarching principles for developing ecological toxicity reference values (TRVs) to perfluorinated compounds (PFCs) along with its implications for risk-based decision-making. Reliable TRVs are critical to evaluate ecological risks for persistent, toxic and bioaccumulative chemicals such as PFCs, but there are no readily available published wildlife TRVs that have been universally accepted. Numerous PFC toxicological studies of varying quality have been published since the early 2000's, but the current state of the science for PFCs research is still very active and studies with new information are continuously being published. A broad review of the literature was conducted in search of toxicity studies for PFCs that could be used to derive dose-based TRVs for birds, mammals, and reptiles. In developing

TRVs for wildlife receptors, studies that measure concentrations in the diet, and then relate that dietary concentration to an effects level are most useful for TRV development. However, there are numerous studies in which PFC concentrations have been measured in liver, blood serum, or bird egg tissue and related to the desired sub-lethal endpoints (e.g., reproductive and developmental). Although these studies are not directly usable as TRVs, they provide critical information regarding toxicity potential and mode of action for various PFCs, relative sensitivities between taxonomic classes to the same PFC or different PFCs, etc. This supporting information was used qualitatively in the decision-making process for selecting dietary TRVs for wildlife, in particular for certain PFCs for which adequate ecotoxicity data were not identified. In many cases, the secondary sources and agency documents reviewed referenced the same few primary articles and each adjusted these primary toxicity data in different ways to generate TRVs. It is important to understand these adjustments when selecting a final TRV for a specific project. The key outcome of this exercise is the development of TRVs that can be considered reasonable candidates for use in ecological risk assessments (ERAs) on a global scale. Until more universally accepted studies are published, these TRVs can be used with an understanding of their associated uncertainties and assumptions. Finally, use of these TRVs to support project-level decision-making for PFOS, PFOA and PFBS is also discussed.

Frequent Fliers: Effects of the Deepwater Horizon Oil Spill to Birds

TP206 Development of oil exposure methods for testing the physiological effects of petroleum crude oil on waterbirds

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During the Deepwater Horizon (DWH) oil spill, many live birds were observed with trace, low and moderate levels of oil on their plumage, but the fate of these birds was unknown. A series of studies were developed to determine the physiological effects of exposure to petroleum crude oil, and its link to hemolytic anemia observed in live oiled birds in field studies during the DWH oil spill. Inherent in study design was determination of appropriate amounts and routes of exposure of avian species to oil. We chose 2 species of piscivorous birds, Laughing Gulls (Leucophaeus atricilla; LAGU) and Double-crested Cormorants (Phalacrocorax auritus; DCCO), to use as models based on relevance to the Gulf of Mexico region, abundance and adaptability to captivity. Previous research in gull chicks orally dosed with 10 mL crude oil/kg daily induced hemolytic anemia after 5 days. Our initial study sought to replicate these findings by gavaging LAGU and DCCO with a 1:1 mixture of artificially weathered DWH oil and catfish fillet slurry to achieve a daily dose of 20 ml/kg body weight for 5 days. The results of this trial resulted in few adverse effects characteristic of exposure to oil. This may have been due to reduced absorption of oil from the gut based on rapid excretion of oily feces. Two subsequent studies were designed to deliver low amounts of oil in food fish throughout the day rather than a single gavage to increase gut retention time and decrease bird handling. Food fish were injected with oil to achieve target doses of 5 or 10 mL oil/kg body weight. Gulls were fed frozen-thawed minnows injected with 200 uL or 400 uL of oil for 28 days. Cormorants were fed live channel catfish fingerlings given an intraperitoneal injection with 2 mL of oil over 21 days. Both species developed clinical signs consistent with oil toxicity induction; however, cormorants more readily accept oil-injected food fish. Lastly, an external exposure study was conducted on cormorants. The breast and back were treated with 13 mL of oil every 3 days for 21 days, applied to cover 20% of the feathered surface area. This method of exposure induced hemolytic anemia, organ abnormalities, and provided evidence of thermoregulatory stress due to greater heat loss in oiled birds. These studies provide information on methods and toxicity effects of multiple routes of exposure of oil to avian species; direct ingestion, indirect ingestion through consumption of exposed prey, and external oiling.

TP207 Development of Methods for Avian Oil Toxicity Studies using the Double Crested Cormorant (Phalacrocorax auritus)

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The majority of previous oil dosing studies conducted on wild-caught birds used gavage methods to dose birds and determine toxicity. However, rapid gut transit time of gavaged oil likely reduces oil absorption. Instead, new oral dosing methods were developed that relied on intra-peritoneal injection of oil into live, sedated feeder catfish for consumption by piscivorous birds to provide better replication of field exposure in the double-crested cormorant (DCCO). Twenty-seven DCCO were collected from Leflore County, MS using boats and a night-lighting capture technique and transported to the National Wildlife Research Center Mississippi Field Station. Upon arrival at the NWRC-MFS, birds were identified by a unique leg band and quarantined in individual 3.3 m x 1.52 m x 2.0 m (L x W x H) concrete floored pens for 14 days in a 23 m x 10 m environmentally controlled building. DCCO were assigned to one of three treatment groups (n=9) to test the toxicity of artificially weathered Deepwater Horizon Mississippi Canyon 252 oil: a control group that was fed fingerling catfish containing no oil, a group that was fed oil-injected catfish that targeted a daily dose of 5 ml oil/kg body weight and a group that targeted a daily dose of 10 ml oil/kg body weight. Clinical signs reported in oil -treated birds included hypothermia, weight loss, lethargy, feather damage, morbidity, and some cases, death. Oral dosing studies identified oil-related toxicity endpoints associated with oxidative stress such as hemolytic anemia, liver and kidney damage, and immuno-modulation or compromise. The results of this study indicate that oral dosing of DCCO with artificially weathered MC 252 oil from the DWH oil spill resulted in clinical signs and changes in a number of hematological, biochemical, and tissue endpoints consistent with petroleum intoxication.

TP208 Avian Toxicity for Laughing Gulls exposed to weathered Deepwater Horizon oil

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Understanding the potential fate of the tens of thousands of birds oiled during and after the Deepwater Horizon (DWH) oil spill is an important goal of the DWH Mississippi Canyon 252 (MC252) Oil Spill Natural Resource Damage Assessment. Consequently, we conducted an avian toxicity study of laughing gulls (Leucophaeus atricilla; LAGUs) exposed to artificially weathered MC252 oil administered in food fish. Study objectives were to determine if orally dosed LAGUs develop clinical signs indicative of hemolytic anemia (Heinz bodies and a decrease in packed cell volume [PCV]), and to determine if there were other responsive endpoints that could be used to indicate injury due to oil exposure. We tested three treatment groups: a control group fed minnows containing no oil, a group fed oil-injected minnows targeting a daily dose of 5 mL oil/kg body weight, and a group fed oil-injected minnows targeting a daily dose of 10 mL oil/kg body weight. Birds were dosed for up to 28 days. Blood samples were taken twice weekly for determination of Heinz bodies, complete blood counts, blood electron microscopy, PCV, hemoglobin concentration, and plasma clinical chemistries. Moribund birds were euthanized and necropsied prior to the end of the study, while the remaining birds were necropsied on day 28. Of the 30 birds tested, 1 of 7 LAGUs dosed with 10 mL oil/kg and 8 of 13 LAGUs dosed with 5 mL oil/kg died or were euthanized within 20 days. Clinical signs reported in oil-dosed birds included lethargy, reduced food consumption, moribundity, and death. PCV was significantly decreased by exposure to oil in a dose-related manner. Transmission electron micrographs confirmed that red blood cells (RBCs) contained Heinz bodies in both dose groups. There were no oil-related changes in plasma clinical chemistry or electrophoresis endpoints with the exception of 3-methyl histidine concentration, which was significantly higher in the 10 mL group compared to the control and 5 mL groups. All of the hepatic antioxidant endpoints measured at necropsy except superoxide dismutase were significantly increased in LAGUs exposed to oil. At necropsy, relative kidney and absolute and relative liver weights were significantly increased in oil-dosed birds. These results indicate oral dosing of LAGUs with artificially weathered MC252 oil from the DWH oil spill resulted in some clinical signs and changes in hematological, biochemical, and tissue endpoints that have been reported in birds exposed to oil.

TP209 Changes in plasma chemistry and organ size in double-crested cormorants after consuming artificially weather DWH oil by contaminated food

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The toxicological effects of oil spills on birds have been studied sporadically over the decades. The devastating effects of oil spills on birds have meant that most rehabilitation centers focus on heavily oiled birds that are clearly distressed and in need of extensive veterinary care. Additionally, there have been few field studies that have focused on toxicological effects other than those directly related to hemolytic anemia, and oral dosing studies of oil are notoriously difficult due to rapid excretion. Methods of oral dosing and external oil application developed for use in the DWH USFWS avian toxicity testing program will add considerably to our knowledge of the toxicological effects of oil on birds. Double-crested (DCCO) were assigned to one of three treatment groups for an oral dosing study: a control group, nominal daily dose of 5 ml oil/kg body weight group and a nominal daily dose of 10 ml oil/kg body weight. Oral dosing occurred by providing live feeder catfish that had been given intraperitoneal injections of oil. The study resulted in statistically significant changes in plasma markers of kidney and liver function, white cell counts, organ weight changes and histological signs of oil intoxication, as well as indications of as yet unidentified markers of oil toxicity in birds, such as cardiotoxicity and effects of clotting processes. DCCO dosed via feed showed severe clinical signs of oil intoxication including hypothermia, weight loss, lethargy, feather damage, morbidity, and some cases, death. This study provided a clear insight into the dose-dependent effects of oil, and how avian health declines as oil ingestion continues. DCCO exposed to high doses via their food source were unable to maintain homeostatic mechanisms and were likely to succumb to oil intoxication.

TP210 Weathered MC252 crude oil-induced anemia and erythropathology in double-crested cormorants (Phalacrocorax auritus) upon oral and dermal exposure

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Injury assessment of birds in the field following the Deepwater Horizon oil spill documented Heinz body formation. However, there is little information about the relationship between route and magnitude of oil exposure and induction of anemia in birds. Here we present two studies that induced anemia in wild-caught birds. In the first experiment, adult mixed-sex double-crested cormorants (DCCOs) were randomly divided into groups and fed oil–injected fish for up to 21 days. In the second experiment, adult, mixed-sex DCCOs had oil (test) or water (control) applied to the breast and back feathers every 3 days, covering approximating 20% of the body surface. Whole blood samples collected during the exposure period were analyzed both by light microscopy using new methylene blue stain and by transmission electron microscopy. Oral and dermal exposure of DCCOs to weatheredDeepwater Horizon crude oil induced hemolytic anemia as indicated by decreased packed cell volume, relative reticulocytosis with an inadequate regenerative response, and presence of degenerate hemoglobin, which is consistent with other reports of oil-exposed birds. Additionally, this study documented extravascular blood loss through hematochezia contributing to the severity of anemia and probable coagulopathy. Ultrastructural assessment of suspected Heinz bodies in birds is recommended as identification by light microscopy is challenging.

TP211 Induction of oxidative stress following light to moderate oral or external oil dosing of double-crested cormorants

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The Deepwater Horizon oil spill exposed tens of thousands of birds to oil. This exposure ranged from heavily oiled birds to lightly oiled birds. While acute mortality was the fate of most heavily oiled birds, the effect of light to moderately oiled birds was not clear. Several of the oiled birds captured in the field were diagnosed with hemolytic anemia through the identification of Heinz bodies in their red blood cells. Heinz bodies are formed through an oxidative mechanism in which hemoglobin sulfhydryl bonding is altered through oxidation resulting in protein denaturation and subsequent aggregation of these proteins. Oxidative stress is a fundamental mechanism by which chemicals can exert toxicity to organisms. Crude oil is a complex mixture of chemicals, including several polycyclic aromatic hydrocarbons which can generate toxic oxygen species upon metabolism. In this study we assessed several liver tissue markers of oxidative stress in cormorants repeatedly exposed either orally or externally to artificially weathered Deepwater Horizon (DWH) Mississippi Canyon 63 252 (MC252) source oil. Significant increases in total glutathione, reduced glutathione and total antioxidant power as well as significant decreases in glutathione peroxidase were observed in both oral and externally dosed birds. Orally dosed birds also showed significantly increased levels of oxidized glutathione and decreased superoxide dismutase activity in liver tissues. These results clearly demonstrate that cormorants exposed to these light to moderate levels of oil either orally or externally are undergoing oxidative stress. This oxidative stress could lead to hemolytic anemia or other detrimental conditions resulting from protein, lipid and nucleic acid oxidation. This project was funded in part by the Deepwater Horizon Natural Resource Damage Assessment Trustees and the Nevada Agricultural Experiment Station.

TP212 AROD and CYP1A Protein Expression in Four Avian Species Experimentally Exposed to Deepwater Horizon Mississippi Canyon 252 Oil

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Four avian species, Double-crested cormorant (DCC, Phalacrocorax auritus), Western sandpiper (WS, Calidris mauri), Laughing gull (LG, Leucophaeus atricilla), and Rock dove (RD, Columba livia), were experimentally exposed to Deepwater Horizon Mississippi Canyon 252 (DWH) oil, orally or applied to feathers, to investigate oil-induced toxicological impacts. Liver and gut tissues were collected for multiple analyses including cytochrome P4501A (CYP1A) enzymatic activity and protein expression. CYP1A is involved in the biotransformation and activation of polycyclic aromatic hydrocarbons (PAHs) found in oil. CYP1A induction is a widely used measure of exposure to specific contaminants including PAHs in avian, as well as laboratory, and other wildlife species. CYP1A enzymatic activity was measured by alkoxyresorufin O-dealkylase (AROD) assays and CYP1A protein expression, as detected by western blot analysis. ARODs demonstrate tissue and species variability in measuring CYP1A activity. We measured activities specific to each of four resorufin ethers: benzyloxyresorufin O-debenzylase (BROD), ethoxyresorufin O-deethylase (EROD), methoxyresorufin O-demethylase (MROD), and pentoxyresorufin O-depentylase (PROD). Enzymatic induction was detected via BROD, EROD, and PROD in livers of DCC orally exposed to 20ml/kg oil as a single dose or for 5 days. Western blot showed a significant increase in CYP1A expression in livers but not in the gut. In a second DCC study (5ml/kg oil or 10ml/kg oil 21 day dosing), all four ARODs were induced in livers as was BROD in the gut. Western blot revealed CYP1A protein induction in the liver. All four ARODs were induced in livers of WS orally exposed to 1ml/kg or 5ml/kg for 20 days. A thermoregulation study on WS after oiling feathers showed enzymatic induction in livers detected via BROD, EROD, and PROD. A wind tunnel study on WS after oiling feathers showed all 4 ARODs induced in livers. 50-mile and 100-mile flight studies in RD after oiling feathers showed only BROD induced in livers in the 50-mile study. CYP1A protein expression was below the western blot limit in WS and RD studies. Hepatic CYP1A activity occurred in all DWH oil-exposed avian species. Further analyses will investigate correlations among these detected changes and other endpoints examined in the birds.

TP213 Gene expression in Seaside Sparrows exposed to oil in Louisiana salt marshes

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Oil from the 2010 Deepwater Horizon oil spill (DWHOS) entered the Louisiana salt marshes food web, where it was incorporated in Seaside Sparrows (Ammodramus maritimus) feathers. In 2011, cytochrome P450 (CYP1A) expression was higher in Seaside Sparrows from oiled sites compared to birds from 'clean' sites, confirming that they had been exposed to oil. While CYP1A expression went down in 2012, the next year birds in both oiled and 'clean' sites had high CYP1A levels compared to 2012, indicating that oil in the sediments was remobilized, possibly by storms. The overall transcriptomic response of birds to oil remains, however, largely unknown. Understanding this response will help us develop sensitive biomarkers of exposure to oil, and clarify the regulation of the CYP1A pathway in metabolism and physiology. In the present study, using microarray techniques we analyzed genome-wide transcriptomic profiles in liver samples from Seaside Sparrows collected in 2011 and 2013 across oiled and 'clean' sites in Barataria Bay, Louisiana. In addition, we compared gene expression profiles in birds that showed high CYP1A levels in 2011 and in 2013, to test whether the transcriptomic response to oil exposure changed with time since the DWHOS. Our study identified genes that are relevant as biomarkers of exposure to oil from the DWHOS or from future oil spills. Our analysis of changes in gene expression across time will contribute to clarifying the persistence of oil from the DWHOS in the affected Louisiana salt marshes.

TP214 How a Single Exposure to Crude Oil Affects Feather Integrity and Flight Performance in Homing Pigeons: A Model for Assessing Migratory Bird Exposures

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External oil contamination of feathers is the most common form of oil exposure to birds, and the acutely lethal effects of heavy external oiling to birds is well known. Many birds died as a result of the Deepwater Horizon oil spill, but the sublethal effects lightly oiled birds may have suffered are not well described. This study utilizes the homing pigeon as a surrogate species for migratory birds to investigate the effects a single external oiling event has on the flight performance of birds. Homing pigeons were equipped with GPS data loggers and released 50 miles from their home loft for a series of baseline and experimental flights. After completion of the baseline flights, MC 252 crude oil was applied to the wing and tail feathers of the birds in the treated group (approximately 20% of their surface area). Repeated experimental flights followed the single application of oil and flight performance parameters were recorded. Data from GPS data loggers found that lightly oiled pigeons flying repeated 50 mile flights took significantly longer to return home and spent more time stopped on route than unoiled birds without changing sustained flight speed. After only a single oil application, feather damage was apparent and increased over time with repeated flight, likely causing changes to flight aerodynamics and requiring the need for compensatory behaviors during flight. These data suggests that birds affected by the Deepwater Horizon oil spill could have experienced long-term flight impairment, inhibiting a bird's ability to sustain flight. This would ultimately decrease the speed of migration and contribute to late arrival dates to desired destinations such as wintering grounds, breeding grounds or stopover sites. Delays during migration have been shown to cause reductions in reproductive success and survival. This work was funded in part by the U.S. Department of Interior's Natural Resource Damage Assessment for the Deepwater Horizon/Mississippi Canyon 252 Oil Spill and the Nevada Agricultural Experiment Station.

TP215 Towards a Physiologically Based Oiling Model (PBOM) for Predicting Thermoregulatory Response and Mortality in Seabirds

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When seabirds are exposed to spilled oil, mortality often results through hypothermia caused by the reduction of feather insulative properties. Some stakeholders in the risk assessment process consider oiling of seabirds to be a non-threshold event, with even the smallest amounts of oil on a bird assumed sufficient to cause mortality. This view is contrary to the prevailing paradigm established in the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) Type A Natural Resource Damage Assessment Model for Coastal and Marine Environments (NRDAM/CME) where a threshold volume of 350 mL is defined as the lethal external exposure threshold for birds. This quantity translates into a threshold oil thickness of 10 µm for spills with diameters over 230 m. Neither approach (i.e., no threshold vs. 10 µm) provides the flexibility to evaluate risk of mortality to seabirds, shorebirds and waterfowl of different body sizes and feeding guilds, or habitats ranging from the tropics to the polar regions. To address this deficiency, we explored the development of a physiologically-based model to predict thermoregulatory response in seabirds after oiling. Here we present: 1) a conceptual model of the physiological response of seabirds to oil exposure; 2) data pertaining to key variables related to avian metabolic response to oiling; and 3) results of preliminary modelling (based on points 1 and 2) from a collaborative effort to establish a Physiologically Based Oiling Model (PBOM) to quantify thermoregulatory responses in seabirds after oiling. We provide and invite discussion on how to incorporate such a model into the ecological risk assessment process to address seabird exposure to spilled oil.

TP216 How has the Deepwater Horizon oil spill changed what we think about the effects of oil on birds?

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Our understanding of the effects of oil on birds has largely been limited to data collected from heavily oiled birds during a spill, embryo toxicity studies and long-term low-dose feeding studies. Deepwater Horizon (DWH) oil spill studies documented free-flying birds with external oil exposure in the vicinity of the spill. Maintenance of feather integrity is fundamental to bird survival, and oil disrupts that integrity. This can rapidly lead to hypothermia and death in cold climates, but under warmer, more favorable conditions, birds will try to remove the oil through preening. Preening can have a two-fold effect: 1) further damage to feathers as the oil is spread over a greater surface area in an effort to remove it and 2) oil toxicity caused by ingestion. The US Department of the Interior-directed DWH avian toxicity studies were designed to improve understanding of the mechanisms of ingested oil intoxication in birds and effects of externally applied oil on flight. More effective and realistic methods for dosing birds were developed as was a more thorough understanding of how oxidative metabolic processes, acting on polycyclic aromatic hydrocarbons, affect avian physiology. It became clear that Heinz body formation and resulting hemolytic anemia are only one type of damage to avian red blood cells. There were also effects on multiple organ systems, immune function, cardiac function, oxidative processes and blood clotting, all of which reduce the overall health of the birds. Additionally, laboratory and field based flight studies showed that even light oiling to wings and tail feathers reduced aerial capabilities in western sandpipers and pigeons. For western sandpipers, flight became costly out of proportion to the mass of the oil applied. They had reduced take off speeds and reduced ability to control flight in a wind tunnel. Homing pigeons flew slower, changed their flight patterns and elevation, and (very importantly for migratory species) changed their flight path. For pigeons in particular, these effects were long-lasting after only a single oiling event because feather damage continued until primary feathers began to be replaced one month after oil application. The combined effects of oil toxicity and feather effects in wild populations have yet to be investigated, but the findings of these studies indicate that even light oiling, as detected in free-flying birds in the vicinity of the DWH spill, may be far more detrimental than expected.

Computational Toxicology: Integrating -Omics and Chemistry to Identify Chemicals of Environmental Concern

TP217 A Practical Guide for Green Molecular Design: Using in silico Approaches To Reduce Toxicological Risk

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Making “greener” chemicals involves more than improving atom economy or working with less solvent, and designing less hazardous chemicals can be difficult for chemists without at least some training in biology and toxicology. This review presents a practical guide to computational tools that a chemist could use early in the molecular design process to spot potential concerns before too much time is invested in synthesis or development. The first part of the guide presents a summary of concepts borrowed from medicinal chemistry and drug development, and how these concepts may be applied to engineer chemicals that are lesslikely to be absorbed or biologically active. Next, we discuss the current milieu of software tools used for drug development and toxicity prediction and the challenges which remain to be overcome in the computational toxicology field. Finally, we provide two workflows using some of the tools deemed most useful, present a case study as an example of how a workflow might be implemented, and conclude by outlining our ideal Green Molecular Design (GMD) tool.

TP218 Exploring comparative oxidative stress toxicity in two common animal models: towards the design of less hazardous chemicals

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Sustainable molecular design of less hazardous chemicals presents a potentially transformative approach to protect public health and the environment. Relationships between molecular descriptors and toxicity thresholds derived by our group previously identified the octanol-water partition coefficient, log P, and the HOMO-LUMO energy gap, dE, as two useful properties in the identification of reduced aquatic toxicity. To determine how these two property-based guidelines need to be expanded when studying chemicals that exert oxidative stress (OS) responses, two common aquatic in vivo models, the fathead minnow (Pimephales promelas) and zebrafish (Danio rerio), were employed to examine lipid peroxidation, DNA damage, total glutathione, and antioxidant gene activation following exposure to 17 different compounds. The first eight compounds included structurally diverse industrial chemicals (bisphenol A, cumene hydroperoxide, dinoseb, hydroquinone, indene, perfluorooctanoic acid, R-(-)-carvone, tert-butyl hydroperoxide) and the second nine compounds included chemicals with SN2 nucleophilic substitution and Michael addition reactivity (3-bromo-1-propanol, (±)-3-chloro-1,2-propanediol, dibromoacetonitrile, glycidol, lindane, sodium decyl sulfate, styrene oxide, tris(2,3-dibromopropyl) phosphate, and tris(1,3-dichloro-2-propyl) phosphate). Overall, acute toxicity and oxidative stress responses varied between fish models and across chemicals. Bisphenol A, cumene hydroperoxide, dinoseb, and hydroquinone ranked highest as inducers of OS. Glutathione was the most consistently affected biomarker, suggesting its utility as a sensitivity response to support the design of less hazardous chemicals. Genotoxicity (changes in nrf2, gclc, gst, and sod) was most significantly ($p < 0.05$) altered due to R-(-)-carvone, cumene hydroperoxide and bisphenol A. Among the SN2 compounds dibromoacetonitrile, tris(1,3-dichloro-2-propyl) phosphate, tris(2,3-dibromopropyl) phosphate, styrene oxide and glycidol were most acutely toxic for both species. Results from the present study indicate that metabolism of parent chemicals and the metabolites resulting in molecular initiation events (MIEs) should be considered during the design of less hazardous chemicals. Moreover, new sustainable molecular design guidelines to reduce OS for electrophilic reactive chemicals (e.g., SN2 nucleophilic substitution, Michael addition reactivity) are being derived by our group.

TP219 In-Silico studies of Organophosphates toxicity with human Acetylcholinesterase by Docking, Site Directed Mutagenesis and Molecular dynamic simulation

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Drinking water is considered a potential pathway for dietary exposure due to pesticide contamination of drinking water sources. Organophophates(OPs) have been reported worldwide in waterbodies including drinking water. OPs have distinct feature of inhibiting Acetylcholinesterase enzyme which is required for hydrolyzing the neurotransmitter (acetylcholine) after the signaling. Docking, site directed mutagenesis and molecular dynamic simulation (MDS) approaches were used to explore mode of binding and inhibition for human acetylcholinesterase (hAChE) and organophosphates (OPs). More than 200 OPs molecules were investigated using Glide docking module of Schrodinger suit as co-crystal structure between two are not available in Protein Data Bank. In initial screening Trp86 was found to be involved in maximum Pi-Cation interaction on anionic subsite of hAChE other than Ser203 (Catalytic site). With extra precision glide docking Phoxim Ethyl Phosphonate (PEP) tops among 200 OPs based on glide docking score and interacted with Trp86, Gly121 and Ser203 whereas MM-GBSA score shows less binding affinity than heptenophos and dichlorovos. Trp86 preferred Pi interaction with ring bearing OPs and hydrophobic interactions with smaller OPs without ring bearing structures. Site directed mutagenesis at Trp86 (Trp86 to Ala86) shown the deterioration of the binding site in terms of size reduction, loss of electrostatic and geometric stabilization in binding cavity and significant reduction in binding of OPs in preferred orientation. Dock score of both wild and mutated hAChE shows a perfect qualitative agreement (R^2=64.1%) towards the study. Molecular dynamic simulation (GROMACS 4.5.5) of hAChE-PEP complex for $4X10^4$ picosecond with SPC16 water system at 310K temperature explained the evident role of Trp86 in stabilizing the ligand at P-site of the enzyme. Asp74 and Tyr124 were noticed in conveying H-bonds. Trp86 have shown consistent and stable distance between residues and ligand. Asp74 and Tyr124 appeared as important residues which contributed H bonds and Ser203 was expected to be closer for interaction to happen as it disappeared during simulation. Study suggests role of Trp86 on binding site is equally important for consideration and infers further investigation. Study also suggests trp86 may play a keyrole in development of more efficient antidotes to overcome the case of human poisoning.

TP220 Differentiating pathway-based toxicity from non-specific effects in high throughput toxicity data: A foundation for prioritizing AOP development

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The ToxCast chemical screening approach enables the rapid assessment of large numbers of chemicals for biological effects, primarily at the molecular level. Adverse outcome pathways (AOPs) offer a means to link biomolecular effects with potential adverse outcomes at the level of the individual or population, thus enhancing the utility of the ToxCast effort for hazard assessment. Thus, efforts are underway to develop AOPs relevant to the pathway perturbations detected in ToxCast assays. However, activity ('hits') determined for chemical-assay pairs may reflect target-specific activity relevant to a molecular initiating event of an AOP, or more generalized cell stress and cytotoxicity-mediated effects. Previous work identified a 'cytotoxic burst' phenomenon wherein large numbers of assays begin to respond at or near concentrations that elicit cytotoxicity. The concentration range at which the "burst" occurs is definable, statistically. Consequently, in order to focus AOP development on the ToxCast assay targetswhich are most sensitive and relevant to pathway-specific effects, we conducted a meta-analysis to identify which assays were frequently responding at concentrations well below the cytotoxic burst. Assays were ranked by the fraction of chemical hits below the burst concentration range compared to the number of chemicals tested, resulting in a preliminary list of potentially important, target-specific assays. After eliminating cytotoxicity assays and other generic xenobiotic response assays (e.g., cytochrome P450 induction and pregnane X receptor activation), the resulting list indicated numerous assays with targets previously identified for AOP development (e.g., thyroid peroxidase, peroxisome proliferator-activated receptor γ, estrogen receptor α, aromatase) along with several novel targets (e.g,, matrix metalloproteinase, thyroid hormone receptorα). Additional analyses identified chemicals that consistently were active within the cytotoxic burst region and compared to predictions of their baseline or narcosis toxicity concentrations. Finally, the analysis of assay responses was used to highlight current ToxCast assays of limited discriminatory ability, which either had zero hits among all chemicals tested or were only activated at cytotoxic concentrations. The contents of this abstract neither constitute nor necessarily reflect USEPA policy.

TP221 Targeted gene expression in zebrafish exposed to chlorpyrifos-oxon confirms phenotype-specific mechanisms leading to adverse outcomes

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Zebrafish models for mild, moderate, and severe acute organophosphorus poisoning were previously developed by exposing zebrafish larvae to chlopyrifos-oxon. The phenotype of these models was characterized at several levels of biological organization. Oxidative stress and mitochondrial dysfunction were found to be involved in the development of the more severe phenotype. Here we used targeted gene expression to understand the dose-responsiveness of those two pathways and their involvement on generating the different zebrafish models. As the severe phenotype is irreversible after only 3h of exposure, we also analyzed the response of the oxidative stress pathway at 3h and 24h. Some of the genes related to oxidative stress were already differentially expressed at 3h. There was an increase in differentially expressed genes related to both oxidative stress and mitochondrial function from the more mild to the more severe phenotype, suggesting the involvement of these mechanisms in increasing phenotype severity. Temporal data suggest that peroxynitrite leading to lipid peroxidation might be involved in phenotype transition and irreversibility.

TP222 Toxicity evaluations in Japanese medaka embryos exposed to 1,2-naphthoquinone with metabolomics

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Oxygenated PAHs (oxyPAHs) are discharged with the exhaust gas from automobile, and generated with photodegradation of parent PAHs in the atomosph ere. They widely distribute in environment including aquatic environment. In previous study, Japanese medaka (Oryzias latipes) embryos were seriously affected exposures of individual oxyPAHs as following; individual oxyPAH specific effects to embryos were caused such as deformation of embryonic yolk, blackened oil globule, mortality, hatching delay, and decrease of hatching rate and induced the malformations on hatched larvae such as incomplete development of the cephalic region and palate, pericardial edema, tubular heart, unabsorbed and hypertrophied yolk sac, and underdeveloped caudal fin. 1,2-Naphthoquinone (NaQ) of 100 mg/L induced the serious malformations on most medaka larvae and that more than 200 mg/L arrested the embryo development and led lethal within a few days from starting the exposure. In this study, we examined the toxicities of 1,2-naphthoquinone in fish embryo using GC/MS metabolomics method, because of exploring the mechanism of that toxicities. Medaka embryos were individually exposed to 200 mg/L and 2 mg/L of NaQs for 6 days, and collected for metabolomics analysis at every day. All metabolome profiles detectable with GC/MS analysis were collected, analyzed with principal component analysis (PCA), and explored the relationship between the effects and the variations of metabolome in embryos. In early embryo development in the first 3 days of exposure, glucose, glucose-1-phosphate, and glucose-6-phosphate were significantly low compared with control group, although the energy is not generated in TCA cycle but mainly glycolytic system. These decrease could cause the development delay. In addition, lactate, which play a critical role in cell differentiation, significantly increased during first 2 days of exposure. This disturbance of lactate might induce the disturbing the embryo development. These abnormal metabolome profiles should deeply relate to the arrest of fish development within a few days from starting the exposure. Ammonia is generally used for the osmotic modulation in fish embryo. Urine significantly increased exposed to NaQ for 5 or 6 days. Then, ammonia resulted to extremely increase in embryos, and affected to hatch larvae as causing malformations.

TP223 Integrating metagenomic sequencing with physicochemical variables to infer historical trends within the cyanobacterial community in freshwater lakes

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Eutrophication has become a worldwide environmental issue for many freshwater lakes and reservoirs. Increased nutrient pollution can contribute to water degradation via the production of harmful algal blooms. This phenomenon has contributed to significant water quality issues for many North American Prairie lakes and potable sources. In addition, long-term monitoring data are often lacking for many aquatic ecosystems and studies are frequently initiated only after serious problems arise. Paleolimnological techniques are often used to reconstruct long-term environmental trends when long-term monitoring data are unavailable. One emerging tool in paleolimnological investigations with potential to accurately track and reconstruct historical trends or changes within the aquatic environment is metagenomic analysis. In this study, sediment cores were collected from a Prairie reservoir, and a combination of physicochemical variables and metagenomic analyses were conducted to investigate trends in algal community composition, identify the presence of potentially harmful cyanobacteria and their toxin-producing genes, and to identify relationships among the phytoplanktic community and various physicochemical variables. Temporal trends in algal community composition were identified through sedimentary phytopigment analyses. Insight into trends in the cyanobacterial community was gained through 16S rRNA sequencing. Finally, a component for the microcystin toxin-producing pathway, the mcyA gene, was identified in sections of the sediment cores and was positively correlated to the abundance of Dolichospermum, a genus of harmful cyanobacteria commonly found in many affected freshwater lakes and reservoirs. Thus, metagenomic analyses in combination with supporting physicochemical characteristics, can provide insight into long-term lake ontogeny and serve as a practical substitute for long-term data where such data is lacking or absent.

Assessing Ecological Risk to Inland Environments Due to Increases in Major Ions

TP224 Timing is everything: assessing the effects of pulse exposure patterns on salt toxicity in Daphnia magna

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Road salts used for deicing roads can runoff and have adverse impacts on freshwater ecosystems. Salt concentrations in aquatic systems, however, are not constant. In Maryland, for example, the flashiness of urban streams translates to pulses of high salinity resulting from periodic runoff events. There is a strong need to better understand the ecological impacts of pulsed exposure patterns of salt in urban freshwater streams as a means to inform road salt regulation. Standard ecotoxicological tests are designed to observe the chronic effects of a chemical stressor at a constant exposure level and therefore provide limited insight into more realistic fluctuating exposures. We aimed to elucidate the effects of fluctuating salt exposure patterns using a 21-day toxicity assay on the freshwater cladoceran, Daphnia magna. Four exposure patterns were used, each controlled for total time and intensity of exposure by using the same time-weighted average. The time-weighted average for all exposures was based off of the reference exposure of 4720 mg/L NaCl for 24 hours; this concentration was the 48-hour LC10. From this time-weighted average, we derived the following exposure patterns: one 24-hour pulse, three 8-hour pulses, or six 4-hour pulses of 4720 mg/L NaCl, and a 21-day constant exposure of 225 mg/L NaCl. We collected data on survival and reproductive endpoints throughout the assay, and measured initial and final lengths. Results strongly suggested that the magnitude of sublethal toxic effect was related to pulse duration and frequency. Shorter and more frequent pulses caused greater detriment to growth and reproduction, suggesting that longer recovery periods between exposures may be important for recovery and energetic homeostasis. Alternatively, survival data suggested a convergence of mortality around 50 percent at day 21, when all treatments reached the same total exposure. The exception to this was the pulse pattern of intermediate duration and frequency (three 8-hour pulses), which had the lowest survival at 20 percent. These data are being used to explore different modeling approaches for predicting lethal and sublethal effects of pulse exposures to salt. Our goal is to develop a model that may be used to address concerns regarding effects of road salt runoff on freshwater organisms. Our results suggest that the influence of pulse exposure patterns must be taken into account when assessing the ecological impact of road salt runoff.

TP225 Establishing baseline exposure histories of fish in watersheds with energy-related resource extraction activity through otolith microchemistry

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The proliferation of unconventional oil and gas wells using high volume horizontal hydraulic fracturing (HVHHF) technology has yielded enormous quantities of highly saline produced waste waters. Releases of these waste waters into surface waters have been known to occur, but in the absence of continuous monitoring of baseline water chemistry, it is difficult to assess exposure to aquatic organisms. Employing effective methods to monitor for exposures to HVHHF waste water and other effluents produced by resource extraction operations (e.g., coal mining) in surface waters is crucial for mitigating the potential risks of these technologies. Teleost fish bioaccumulate ambient metals in otoliths throughout their life history and thus represent potentially valuable tools for tracking metal exposures associated with energy-related resource extraction processes. Accessing this exposure history through laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS), we hope to reconstruct and discriminate the exposure histories of fish from watersheds that contain various energy-related resource extraction activities. Otolith microchemistry will be compared between fish sampled from watersheds with no resource extraction activity and those from watersheds with resource extraction activities to determine a baseline presence-absence signal. Additionally, otoliths will be compared between fish in watersheds that have either HVHHF or coal mining activity. For example, Li, Sr, and Ba have been associated with waste waters produced by HVHHF and would be a likely biomarker in the otoliths of fish exposed to these waters, while a different set of metals would be detected in otoliths of fish exposed to acid mine drainage or coal mine effluents. Fish will be sampled in watersheds with and without resource extraction activities (e.g., HVHHF, coal) in Ohio or West Virginia during summer 2016 to establish baseline otolith microchemistry and preliminary results will be presented. Trace element analysis of otoliths may provide a method for tracking exposures of fish in watersheds experiencing changes in water quality connected to energy-related resource extraction.

TP226 Effects of Winter Road Salt Application and Episodic High Conductivity Mesocosm Pulses on Southern Appalachian Headwater Stream Macroinvertebrates

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Freshwater organisms are at risk from exposure to elevated levels of salts due to runoff following winter road salt applications. Climate records of winter snow falls were compared to data gathered every 15 minutes from In Situ water monitors installed at the ouflow of seven headwater sub-basins of the Upper South Fork of the New River (USFNR) by the AppAqua research cluster. Data displays mean background specific conductivity levels (over a five year period, 2010 – 2015) of 30 – 50 uS/cm at reference sites, 140 – 185 uS/cm at moderately impacted sites, and 610 – 740 uS/cm at highly impacted sites. While episodic high conductivity pulses during/after winter storms reach levels as high as 7,000 – 12,000 uS/cm (equivalent to salinities of 6.6 – 11 ppt) in the urbanized stream catchments. A negative correlation exists between long-term waterhsed specific conductivity (SC) trends (monitored during 2010 – 2015) and macroinvertebrates NCBI stream health values (calculated with Family and Genus tolerance values) along with Family and Genera richness (r^2 of 0.7306, 0.5999, 0.6366, and 0.6114 respectively). To determine the salt tolerance of benthic macroinvertebrates to these short term episodes of high road salt levels, acute toxicity tests were performed with a focus on one sensitive Family [Heptageniidae: tolerance value (TV) of 2.44 on a scale of 10] and two sensitive Genera [TV of 0.5 to 1.33 on a scale of 10] of Ephemeroptera at a concentration of 10,000 uS/cm [SC values used for mesocosm exposures were determined from analyzing the water monitoring data] for up to 96 hr with varying rest periods between trials (23, 18, 12, 6, and 0 hrs). Two exposure methods were utilized: 1) baseline results – macroinvertebrates returned to reference water (< 100 us/cm) post – exposure, 2) pulse results – macroinvertebrates returned to water that mimic background SC levels that occur between storm events. When utilizing exposure method two, salt induced mortality from acute salt exposure begins to occur during an exposure of 1 hr on at 10,000 uS/cm and 23 hr off at 900 uS/cm. An LC_{50} response was first measured during 6 hr on/18 hr off after 96 hr. When comparing 6 hr on/18 hr off with 12 hr on/12 hr off, no statistical difference was observed, but once compared to the longer time duration pulse exposures, a statistical difference is evident (i. e. 12 hr on/12 hr off vs. 18 hr on/6 hr off).

TP227 Evaluation of spatial and temporal responses of multiple aquatic taxa to stressors related to surface mining

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The Powell River (Virginia and Tennessee, USA) is a biologically diverse ecosystem, supporting several species of threatened and endangered fish and freshwater mussels. Coal mining is extensive in the upper watershed, with 28% of land area in active surface mining permits and 72% of the underground area in active and abandoned deep mines. In the upper Powell River, elevated concentrations of major ions and associated measurements of total dissolved solids and specific conductance have been attributed to surface mining activities. In this study, we examined recent (2000-2015) and historical data (1963-1999) on biological communities, including macroinvertebrates, fish, and freshwater mussels, to determine their spatial and temporal relationships with surface mining, specific conductivity, and other stressors in the tributaries and mainstem of the upper Powell River. Evaluation of recent data illustrates widespread impairment of macroinvertebrate communities in Powell River tributaries, with a negative relationship between the presence of Ephemeroptera taxa and measured specific conductivity as well as the proportion of the watershed in surface mining permits. Mining-influenced tributaries and their receiving streams also have an impaired fish community, with a general predominance of tolerant species and omnivores and few darter species and insectivores. In the mainstem Powell River, condition of the macroinvertebrate, fish, and freshwater mussel communities improves with distance downstream from surface mining operations. However, evaluation of historic data showed that impairment of aquatic communities in the tributaries and mainstem of the Powell River began more than 40 years ago, before the rapid increase in surface mining and associated major ion concentrations that occurred in the 1990s. We will discuss methodology used to discern effects of major ions relative to other stressors on various taxa, the limitations of these methods, and relevance to ongoing efforts of regulation and restoration.

TP228 Stream periphyton responses to mesocosm treatments of equal specific conductance but different major ion contents measured with in situ fluorometry

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A stream mesocosm experiment was designed to compare biotic responses among streams exposed to an equal excess specific conductivity target of 850 µS/cm relative to a control that was set at 200 µS/cm. Three treatments were based on dosing the background mesocosm water, a continuous flow-through mixture of natural river water and reverse osmosis treated water, with stock salt solutions prepared from 1) a mixture of sodium chloride and calcium chloride (Na/Cl chloride), 2) sodium bicarbonate, and 3) magnesium sulfate. Here we focus on comparing stream periphyton communities across treatments based on measurements obtained from a Pulse-Amplitude Modulated (PAM) fluorometer. The fluorometer is used in situ and, with built in algorithms, distributes the total aerial algal biomass (µg/cm2) of the periphyton among cyanobacteria, diatoms, and green algae. Eight locations within each of the 1 m2 (0.3 m W x 3.33 m L) mesocosm gravel sections were assessed approximately every other day throughout the dosing period. The fluorometer based assessment showed all mesocosms were statistically similar in terms of algal periphyton before dosing began. Changes in the community began as early as 2 days into dosing and were statistically significant by 1 week of dosing, with the magnesium sulfate treatment exhibiting greater cyanobacteria biomass relative to the control and the sodium bicarbonate and Na/Ca chloride treatments. This effect persisted throughout 28 days of dosing. Diatoms biomass was significantly greater in the Na/Ca chloride treatment and the sodium bicarbonate treatment than in the control and magnesium sulfate treatment. Total algal biomass was greater in the magnesium sulfate treatment compared to the control and Na/Ca chloride treatments, but not until 28 days. These results suggest that the ca. 834 µS/cm conductivity treatments did not significantly affect stream periphyton biomass until 28 days of continuous dosing and only for the magnesium sulfate treatment. However the major ion content of the elevated conductivity can be particularly important to controlling the relative abundances of cyanobacteria and diatoms. This effect is realized relatively quickly and this could translate to important differences in the food source for grazers in the systems. Changes in food quality may explain why the total periphyton biomass elevated later in the experiment, because cyanobacteria are not a favored food source compared to diatoms.

TP229 Chronic Salt Impact on Mayflies down under

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The stream macroinvertebrate orders Ephemeroptera (mayfly), Plecoptera and Thricoptera (EPT) species are known for their sensitivities to changes in water quality and are thus widely used in biomonitoring studies. Species Sensitive Distribution (SSD) of the acute lethal sensitivity of stream macroinvertebrates exposed to salinity suggest no adverse effect at 1 mS/cm. Instead, a 50% reduction in EPT species has been seen in south-east Australia at 1 mS/cm. Suggesting either (a) the current protection limit of 1 mS/cm for effluent discharges is not achieving “no adverse effects” to the environment or (b) other factors confounded with salinity are causing the loss of EPT. To resolve which of these hypothesises we aim to determine sub-lethal effects of salinity with chronic exposures, using Ephemeroptera. Growth rates and generational effects will be investigated to determine sub-lethal effects from low salinity exposures. Ephemeroptera species in Australia are not known to be parthenogenetic, providing difficulties in culturing methodology. This data will aid in forming a true representation of what the guidelines should be achieving and if sub-lethal effects are occurring, explaining the reduction in ETP species in low effluent salinity areas.

TP230 Relationships between water mineralization and electric conductivity of natural waters in the Aral Sea Basin

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Salinization of surface waters as a consequence of re-introduction of water from irrigated fields and industrial effluent is a common problem in plain areas within Aral Sea Basin (ASB) with negative effect on aquatic ecosystems and human health, mostly in lower reaches of rivers. Many water quality standards for human consumptions around the world, including the ASB region, are set at 1 g/L (total ions content). There was no trend in salinity levels between 1913 (when measurements began) and he 1960’s where salinity average annual salinity levels were between 0.42-0.62 g/L. In the 1960’s, salinity started to rise reaching 0.92-1.12 g/L between 2005 and 2014. The objective of the present work was to analyse ionic composition, dynamics of water salinization (WS) and its relationship with electric conductivity (EC, µS/cm) in the main rivers of the ASB – Amudarya (AD) and Syrdarya (SD). We used data from the State Hydrometeorological Service of Uzbekistan for 2005-2014. In upper reaches of main rivers: Amudarya (AD) and Syrdarya (SD) the total water mineralization usually does not exceed: 0.5 g/L in the period May – October and 0.8 g/L in the period November – April. The dominant ions in upstream of rivers are: AD – sulphate? hydrocarbonate? chloride? calcium? sodium? magnesium and SD – hydrocarbonate? sulphate? chloride? calcium? sodium? magnesium. However, in midstream and downstream of both rivers dominant ions are: sulphate? chloride? hydrocarbonate? sodium? calcium? magnesium. There were strong linear relationships between salinity and EC with r^2 values of 0.91-0.99. Mean values of salinity and EC for the study period (2005-2014) were 0.65 g/L and 916 µS/cm; for the upper AD reach; 1,12 g/L and 1644 µS/cm for the lower AD reach; 0.61 g/L and 925 µS/cm for SD upper reach; and 0.97 g/L and 1375 µS/cm for SD middle reach. Thus, in AD 1 mg of salinity was equal to 1.41 µS/cm in the upper reach and to 1.47 µS/cm in the lower reach. However, in SD 1 mg of salinity was equal to 1.52 and 1.42 µS/cm in the upper and middle reaches, respectively. This is most likely explained by considerable variations in ionic composition of water. Preliminary results reveal that the water quality standard of 1 g/L in ASB basin will be reached when EC equal to about 1410-1520 µS/cm depending on the river basin.

A SETAC Pellston Workshop® on Environmental Hazard and Risk Assessment Approaches for Endocrine-Active Substances Data

TP231 Challenges in Assigning Endocrine Specific Modes of Action: Recommendations for Researchers and Regulators

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As regulatory programs focus on evaluating substances for their endocrine disrupting properties, there is a need for careful study design and data interpretation to distinguish between endocrine versus non-endocrine specific responses. This is particularly important where specific criteria are under development to identify endocrine disrupting properties to enable hazard-based regulation. Irrespective of the regulatory process, most jurisdictions use the WHO IPCS definition of an endocrine disruptor (ED), requiring that a substance is demonstrated to cause a change in endocrine function that consequently leads to an adverse effect in an intact organism. Such a definition is broad, and at its most cautious, might capture many mechanisms that in general would not specifically be considered ED. For instance, stress is a non-specific, neuro-endocrine response that can lead to adverse outcomes. In addition, non-endocrine toxic mechanisms (e.g. hepatotoxicity, acetylcholinesterase inhibition) may operate secondarily or in parallel to impact the endocrine system and apical endpoints downstream. Furthermore, endocrine responses may be adaptive in nature and designed to maintain homeostasis rather than inducing an irreversible adverse effect. The likelihood of indirect effects is increased in (eco)toxicological studies requiring the use of maximum tolerated dose levels, which must produce some adverse effect. The misidentification of indirect effects as truly ED has serious consequences in terms of triggering animal and resource intensive testing and potentially severe regulatory consequences. This poster will expand on the issues and recommendations made in the oral presentation of a review, based on 6 case study substances, conducted to evaluate scenarios that could complicate the assessment of whether or not a substance is an endocrine disruptor. Various lines of evidence were identified that can be considered when evaluating if an adverse effect is causally linked to a primary endocrine system interaction. A weight of evidence approach was used to evaluate available data for the case-study substances. In this approach, the weight of evidence was based on biological plausibility, empirical support, and essentiality of key events in adverse outcome pathways to determine whether an endocrine mode of action can be conclusively assigned to the effects observed for a given substance.

TP232 Uncertainties in biological responses that influence hazard or risk approaches to the regulation of endocrine active substances

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Endocrine Disrupting Substances (EDSs) may have certain biological effects including delayed effects, multigenerational effects, and non-monotonic dose response relationships (NMDRs) that require careful consideration when determining environmental hazards. The case studies evaluated for the SETAC Pellston Workshop™: Environmental Hazard and Risk Assessment Approaches for Endocrine-Active Chemicals and other key examples from the literature are discussed in this poster and we summarize the key issues and examples presented in the platform session. EDSs can have specific and profound effects when exposure occurs during sensitive windows of the lifecycle (development, reproductive). This creates the potential for delayed effects where the adverse effect becomes manifest when exposure has ceased, possibly in a different lifestage. This underscores the need for testing in appropriate (sensitive) lifestages and full lifecycle designs that capture adverse effects wherever they occur in the lifecycle. Such tests are available in the tool box and should be employed to derive endpoints that can be considered protective of all life stages. Similarly, the potential for effects to be manifest in subsequent generations (multigenerational effects) has also been raised as a potential issue in the derivation of appropriate endpoints for EDSs. However, the evidence for such effects beyond the second generation is limited. Indeed this is reflected in the design of new higher tier tests to assess endocrine active substances (EASs) developed by the OECD and US-EPA that move to extended one-generation designs and away from multi-generational studies for fish and mammals. The occurrence of non-monotonic dose or concentration response relationships is also considered a limiting factor for reliable risk assessment of EDSs. Substantial data reviews are underway to inform on their occurrence. However, evidence to date indicates they are more prevalent in in vitro and mechanistic data, not often translating to adverse apical endpoints that would be employed in risk assessment. A proposal of how to evaluate NMDRs in the context of endocrine hazard and risk assessment procedures is presented. If careful consideration of delayed, multigenerational and NMDR effects are made, it is feasible to assess environmental endocrine hazards and derive robust apical endpoints for risk assessment procedures ensuring a high level of environmental protection.

TP233 Current limitations and a path forward to improve testing for the environmental assessment of endocrine active substances

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To assess the hazards and risks of possible endocrine active chemicals (EACs), there is a need for robust, validated test methods that detect perturbations of endocrine pathways and provide reliable information for evaluating potential adverse effects on apical endpoints. One issue of significant concern for current EACS screening/testing programs involves resources such as cost, time, trained personnel, and animal use. This is especially problematic when considering the number of chemicals that some regulatory authorities need to assess. One way to address this challenge is to prioritize chemicals for possible in vivo testing by using in vitro high throughput (HTP) assays focused on a suite of endocrine molecular initiating events (MIEs). Additional challenges associated with the design and conduct of in vivo EAC screening and testing include the selection of appropriate species (i.e., sensitive and amenable to laboratory testing), endpoints and life-stages. A component of this involves experience gained in the use of existing tests to determine, for example, assays that have demonstrated exceptional sensitivity to perturbation of a given MIE. However, the strategic use of HTP data and/or early screening level information may help guide the selection of existing assays that can further evaluate a given EAC modality. Further challenges for EAC screening and testing involve guidance and optimization in several areas, such as concentration setting, statistical power to detect biologically significant adverse effects, delivery and analytical measurement of test substances, availability of technical expertise, and study interpretation, including linking mechanistic to apical effects. Some of these areas can be addressed by the lessons learned and best practices developed through recent experiences conducting EAC screening/testing. Additionally, the collective assessment of EAC screening and testing data (e.g. compilation of historical control data) can be leveraged to refine test designs and performance criteria to maximize the power and utility of EAC screening/testing. Finally, a number of recommendations are provided for longer term research to address areas of uncertainty, including identifying potentially sensitive species for which test methods currently do not exist (e.g., invertebrates) and key endocrine pathways in addition to estrogen, androgen and thyroid signalling.

TP234 Assessing the Effects of Endocrine-active Substances on Wildlife Populations: A Case Studies Summary

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For ecological risk assessment, endocrine disruptors require the establishment of an endocrine mode of action (MoA) with a plausible linkage to a population-relevant adverse effect. Current ecotoxicity test methods mostly incorporate apical endpoints although some also include mechanistic endpoints, at the subcellular through organ level, which can help establish an endocrine MoA. However, the link between these endpoints and adverse population-level effects is often unclear. In preparation for a 2016 Pellston Workshop® on Environmental Hazard and Risk Assessment Approaches for Endocrine-Active Substances (EAS), several case studies of EAS (tributyltin, ethinyl estradiol, perchlorate, trenbolone, propiconazole, and vinclozolin) were used to evaluate the population relevance of toxicity endpoints in various taxa. For some taxa, like mollusks, the population relevance of tributyltin-induced imposex is well established. However, for other taxa, the population relevance of observed effects is not as well understood. Furthermore, potential adaption and recovery processes also are important to consider when evaluating the adverse effects of EAS on wildlife populations. As our understanding of endocrine perturbations and key event relationships improves, adverse population level effects will be more easily and accurately predicted. This poster presentation expands on a platform presentation on the topic of assessing the effects of EAS on populations and will focus on examples of potential population relevance from the case study chemicals as well as briefly discuss future needs to better predict population level effects.

TP235 Evaluating the Credibility of Histopathology Data in Environmental Endocrine Toxicity Studies

J.C. Wolf, Experimental Pathology Labs., Inc.; G. Maack, Federal Environment Agency / Ecotoxicological Assessment

Agencies responsible for environmental protection are tasked with developing regulatory guidance that is based on the best available scientific evidence. Histopathology is a common endpoint in toxicologic bioassays; however, because of the subjective nature of this endpoint, and the advanced level of specialized training required for its effective utilization, the reliability of histopathology data can be inconsistent. For the present study, a total of 189 papers that involved investigations of endocrine active substances (EAS) were reviewed for the credibility of their reported histopathology findings. The review process incorporated standardized criteria for article and data selection, and systematic procedures for data evaluation and histopathology credibility (HC) scoring. A major outcome of these efforts was the finding that 54% of the examined papers contained histopathology data that were considered to be either highly credible or credible, whereas such data were deemed to be of equivocal, dubious, or no credibility in 46% of cases. The ultimate goals of this work are to draw attention to reliability issues that can affect the histopathology endpoint, provide recommendations to improve the quality of this endpoint, and suggest an approach for the expeditious and judicious use of histopathology data in weight of evidence determinations required for hazard and/or risk assessment. This exercise was conducted initially as part of a SETAC-Pellston Workshop™ entitled "Environmental Hazard and Risk Assessment Approaches for Endocrine-Active Chemicals (EHRA): Developing Technical Guidance Based on Case Studies to Support Decision Making" that was held in Pensacola, Florida, USA, from January 31st to December 5th, 2016.

Regulatory Directions – Poster Only

TP236 A strategic approach to fulfilling data gaps for environmental risk assessment of iso-alcohols

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Environmental risk assessments require quality data to provide defensible environmental quality benchmarks. Quantitative Structure Activity Relationship (QSAR) endpoint estimates are often appropriate for alcohols with a very strong correlation to aquatic toxicity test data. However, QSAR estimates require comprehensive justification to demonstrate applicability, and still may not fully meet regulatory requirements, leading to extensive long-term toxicity testing. Here, limited, strategic environmental testing was used to support QSAR predictions, thereby reducing animal testing while still meeting regulatory requirements. Aquatic toxicity testing with algae, daphnids and fathead minnows (OECD 201, ISO 20665 and OECD 210) was performed with isooctanol and isoundecanol. The study objective was to employ a testing program consisting of long-term fish (limit test), invertebrate and algal toxicity tests to demonstrate that QSAR estimations accurately predict aquatic effects from long-term continuous exposure to these substances, further supporting the use of QSAR models across a range of iso-alcohols. The data demonstrate that the QSAR model employed accurately characterized the hazard of iso-alcohols and is protective of these endpoints. Moreover, this combined information, by demonstrating a regular and predictable pattern of toxicity amongst these substances, further justifies read-across between substances for other endpoints (such as bioaccumulation) and supports efficient use of data for general purpose risk assessments.

TP237 Development and application of a density dependent matrix population model for Atlantic killifish (Fundulus heteroclitus)

D.H. Miller, USEPA / Midcontinent Ecology Division; B. Clark, D.E. Nacci, USEPA / Atlantic Ecology Division

Ranging along the Atlantic coast from US Florida to the Maritime Provinces of Canada, the Atlantic killifish (Fundulus heteroclitus) is an important and well-studied model organism for understanding the effects of pollutants and other stressors in estuarine and marine ecosystems. Matrix population models are useful tools for ecological risk assessment because they integrate effects across the life cycle, provide a linkage between endpoints observed in the individual and ecological risk to the population as a whole, and project outcomes for many generations in the future. We developed a density dependent matrix population model for Atlantic killifish by modifying a model developed for fathead minnow (Pimephales promelas) that has proved to be extremely useful, e.g. to incorporate data from laboratory studies and project effects of endocrine disrupting chemicals. We developed a size-structured model (as opposed to one that is based upon developmental stages or age class structure) so that we could readily incorporate output from a Dynamic Energy Budget (DEB) model, currently under development. Due to a lack of sufficient data to accurately define killifish responses to density dependence, we tested a number of scenarios realistic for other fish species in order to demonstrate the outcome of including this ecologically important factor. We applied the model using published data for killifish exposed to dioxin-like compounds, and compared our results to those using a previously published stage-based density-independent killifish matrix model. By considering how models can accommodate variation in life histories and account for ecological factors such as density dependence, we can more easily develop other fish species-specific matrix models to characterize population status and predict more realistically the ecological impacts of stressors.

TP238 Ecological Risk Assessment of Complex Substances (UVCBs) using a holistic approach: A Case Study with Resins and Rosins

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Ecological risk assessment of complex multi-component substances (e.g. UVCBs – Unknown or Variable Composition, Complex Reaction Products or Biological Materials) pose a challenge to regulators due to these substances' variable and often unknown composition, including both identity and quantity of chemical species present in the UVCB substance being evaluated. Furthermore, there are additional challenges in accurately matching of test data to the substances registered and being assessed by the regulator. Approximately 300 organic UVCBs remain to be addressed in Canada as part of the next phase of the Chemicals Management Plan (CMP). The approach taken for assessing UVCBs is to consider multiple lines of evidence in a weight of evidence approach when assessing a substance's potential to cause harm under the Canadian Environmental Protection Act, 1999. More specifically, in order to evaluate fate, exposure and effects of a UVCB within our ecological risk assessment, data for the whole UVCB substance (e.g., whole-substance toxicity tests) and data available on individual constituents, together with reasonably accurate compositional information, is usually needed as reliable whole substance data for all end-points of interest are often not available. Thus, there is a requirement to understand how significant a contribution each major component may have in the UVCB to the endpoint or property being evaluated, as well as, determine if component data provides sufficient evidence and weigh the evidence accordingly to conclude on environmental risk for the whole UVCB substance. In this way, all reliable and available information is used in order to conduct the most accurate, robust and comprehensive assessment possible. Here we illustrate this approach using a case study for the assessment of four resins and rosins (HR, HRPE, HRGE and HRTE) published under the first phase of the CMP (2006-2012) known as The Challenge. Resins and rosins are high volume organic substances that are used in Canada for various purposes, which may include application in adhesives and sealers, cosmetics, electronics, paints and coatings, and inks and paper. A summary of the analysis of substance identity, physical-chemical properties, environmental fate and exposure, biological effects and ultimately ecological risk outcome will be presented for these four substances.

TP239 Progressing from Alternatives Assessment to De Novo chemistry: Part II

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Presently selection of functionally equivalent chemicals is mainly limited to Alternatives Assessment. As Green Chemistry based de novo design progresses, Alternatives Assessment will gradually transition to functionally equivalent de novo chemicals as alternatives to existing chemicals. Recent TSCA legislation indicates that future new chemical regulations will be partially driven by alternatives assessment as well as hazard and exposure screening (which is actually risk screening). Such a screening phase would be followed by an appraisal of all available data for a more detailed hazard/exposure/risk assessment. More detailed information might also allow for a life cycle assessment screening. Currently "Read-across" is used as the main available technique for filling data gaps, but QSAR, toxicogenomics, computational chemistry, molecular toxicology and big data analytics are leading to more powerful solutions. These same techniques inform de novo design and increase the size of the modeling database, thereby reducing uncertainty, hence increasing reliability.- If only for regulatory purposes, chemicals based on both Alternatives Assessment and de novo design-all go through a detailed hazard and exposure assessment phase. When minimizing hazard, a principle inherent to de novo design, risk is simultaneously decreased depending on the exposure assessment (as risk=hazard*exposure.) Exposure assessment can provide information to prioritize the hazards and hence the risk. Functionally equivalent alternatives that fail the screening and assessment phase(s) will require a return to R&D to design other functional equivalents, with most likely different structural features. Going forward, de novo design and alternatives assessment will thus provide a convergent pathway to attaining sustainability.

TP240 Updates to the proposed Canadian regulatory framework for the environmental assessment of new active ingredients in human and veterinary drugs

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A Canadian regulatory framework has been developed specifically for active ingredients in human and veterinary drugs regulated by the Food and Drugs Act (F&DA) to assess risks to the environment and to human health resulting from environmental exposure. This regulatory framework has been designed to harmonize with the drug approval process stipulated by the F&DA and its regulations. Health Canada developed this framework in collaboration with stakeholders including Environment and Climate Change Canada, industry and environmental non-governmental organizations. The framework was endorsed in principle by all stakeholders in 2011. Since then, the proposed regulatory framework has been revised to increase alignment with environmental assessment approaches in other jurisdictions and incorporate recent technical developments related to the environmental assessment of active ingredients in human and veterinary drugs. Highlights include a proposal to adopt VICH guidelines 6 and 38 for environmental assessment of active ingredients in veterinary drugs, and updates to screening level exposure assessments such as new Canada specific defaults for predicting environmental concentrations of veterinary drugs in soils. The proposed changes will be the subject of an upcoming stakeholder consultation. The purpose of this poster is to present the revised regulatory framework being proposed and generate feedback on the updates.

Aquatic Toxicology and Ecology – Poster Only – Part 2

TP241 Characterization of Copper Residuals in Sediments of a Freshwater Reservoir Following Copper-citrate and -gluconate Algaecide Treatments

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Copper algaecide applications provide unique opportunities to ask questions about copper residuals following pulse exposures in aquatic systems. Following applications of copper citrate and gluconate algaecide in a reservoir, copper residuals in sediments were characterized in terms of factors (i.e. concentration and form) that influence risks to benthic invertebrates. The specific objectives of this experiment were to 1) predict and measure "total" sediment copper concentrations from an algaecide application, 2) measure concentrations of ligands in sediments and copper availability and 3) measure copper concentrations necessary to elicit adverse effects for an amphipod (Hyalella azteca) in 10d laboratory toxicity experiments. Comparison of copper concentrations using a mass balance model (assuming complete partitioning of copper from the aqueous to sediment phase) to measured concentrations in situ assessed the relative influence of fate processes in post-treatment sediment copper concentrations. Predicted concentrations overestimated measured concentrations by approximately 20 times, indicating the influence of dilution and dispersion on copper partitioning to sediments. To measure copper form, multiple lines of evidence were used. Ligands including organic matter concentration (OM), acid volatile sulfides and particle size distribution (PSD) were quantified. Two divergent sediments (based on OM and PSD) were used to bound the sediment types at this site. The sediments were amended with a series of concentrations of the algaecide. Correlations between copper amendment concentrations and copper concentrations sequentially extracted from sediment fractions were used to identify ligands available for binding copper. Sediment toxicity experiments with second instar H. azteca were used to measure the mass of copper from a pulse-exposure eliciting bioavailable copper species. For the silt dominated sediment with high OM (2.35%), oxidizable and residual fractions were correlated with amended copper (R^2=0.98 and 0.85 respectively). For the sand dominated sediment with low OM (0.46%), carbonate, reducible and oxidizable fractions were correlated with increasing copper amendments (R^2=0.84, 0.81 and 0.80 respectively). LC_{50s} ranged as a function of sediment type [444 (367-521 mg Cu/kg) and 46 (31-60 mg Cu/kg]). These results suggest the influence of in situ exposure modifying factors in decreasing concentrations and toxicity of copper from algaecide inputs.

TP242 Probabilistic Risk Evaluation of Metals to Aquatic Organisms Using Species Sensitivity Distribution

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The water quality criteria (WQC) in South Korea is not appropriate to protect aquatic organisms from the exposure of metals. The continuous discharges consisting of metals into the water environment have been occurred due to human activities, so the environmental concentration and the risk of organisms should be observed. In the present study, the exposure and sensitivity of aquatic organisms by metals were examined. The study focused on domestic species and four metals including copper, cadmium, zinc, and lead. Species sensitivity distributions (SSDs) and environmental concentration distributions (ECDs) were established using toxicity data and monitoring data, respectively. For each metal, hazardous concentration affecting 5% of species (HC5) was calculated and both distributions were compared. In addition, the distributions were combined to one curve depending on probability. In result, we found that the Korea water environment is rarely risky to aquatic organisms for cadmium and lead. However, the organisms were not considered to be safe with copper and zinc because some of organisms could be exposed to the metals in the environment.

TP243 Acute toxicity of waters from the Rio Doce Basin after the breakage of a major tailings' dam

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In November 5th 2015, a major tailing dam was broken and released more than 60 million of cubic meters of residues and mud to the close water bodies. The "mud wave" destroyed the downstream districts located in the states of Minas Gerais and Espirito Santo, killed 16 people, and travelled about 600km through the Rio Doce Basin until reach the Atlantic Ocean and spread along the coast. The disaster immediately caused massive mortalities of fish and invertebrates, and turned waters into brownish orange color due to the presence of suspended fine particles. This study is part of an independent initiative involving citizen science that aims to evaluate the impacts of this disaster; and in this case the main objective was to assess the water toxicity in the affected areas. Fourteen water samples were collected along the Rio Doce Basin, includig the rivers Doce, Carmo and Gualaxo do Norte, and tested for acute toxicity to Daphnia similis. Raw and 4h-aerated samples were tested. The raw samples were not considered toxic after 48h exposure, while 8 aerated samples were considered toxic to D. similis. Worst toxicities occurred in the samples collected closer to the broken dam. We compared our results with the official reports on the water chemistry, and the concentrations of metals were high in most samples and exceeded the Brazilian standards for As, Cd, Pb, Cr, Mn, Ni and Se, which could explain, at least partially, the observed toxicities. The effects of aeration on the toxicity may indicate that in field, natural conditions would allow the exposure to metals and negative effects on the local biota. The analyses showed that the waters from the Rio Doce Basin are potentially toxic, possibly due to release of contaminated residues from the broken dam.

TP244 EPA's 2016 Updated National Cadmium Ambient Water Quality Criteria for the Protection of Freshwater and Estuarine/ Marine Aquatic Life

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EPA's National Recommended Water Quality Criteria for aquatic life were first developed for cadmium in 1980 and last updated in 2001. Consistent with provisions in the Clean Water Act, National Recommended Water Quality Criteria are periodically revised to ensure they incorporate the latest scientific information and are protective of aquatic life. Substantial additional toxicological data have become available since 2001, and these data were used to support development of the 2016 revised criteria. The final updated criteria collectively incorporate new toxicological data for an additional 75 species and 49 genera and now encompass 75 freshwater genera for acute toxicity (compared to 55 genera in the 2001 criteria), 20 freshwater genera for chronic toxicity (compared to 16 genera in the 2001 criteria), and 79 estuarine/marine genera for acute toxicity (compared to 54 genera in the 2001 criteria). Although additional toxicological data were not available to directly derive an estuarine/marine chronic criterion, the acute-to-chronic ratio was revised using data for a broader range of marine and representative freshwater species to provide a more robust revised criterion value. The final updated criteria also incorporate additional toxicological data that are protective of federally threatened and endangered species, most notably salmonids. A summary of the toxicological data and approach used to derive the revised criteria is presented, along with final criteria values that were published in March 2016.

TP245 Influences of pH changes in the boundary layer on Cd bioavailability to Chlamydomonas reinhardtii

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Over the last 30+ years, a general consensus has emerged that the best predictor of the biological uptake of trace metal cations by phytoplankton is normally the concentration (or activity) of the free metal ion. In the present study, we observed that at a fixed free Cd^{2+} concentration, the short-term Cd uptake rate by nitrate-acclimated Chlamydomonas reinhardtii was significantly lower in a cysteine-buffered medium than in anitrilotriacetic acid (NTA)-buffered medium. However, when the experiments were repeated with ammonium-acclimated C. reinhardtii, there was no significant difference in the Cd uptake rate between the cysteine- and NTA- buffered media. Because the free Cd^{2+} concentration and pH remained constant in the bulk solutions, the lower Cd bioavailability in the presence of cysteine does not appear to be associated with chemical changes in the bulk exposure media. On the other hand, in a poorly-buffered medium we observed that the extracellular pH increased progressively over time for nitrate-acclimated cells but not for the ammonium-acclimated ones. A simulation of Cd speciation in the cysteine- and NTA-buffered media with MINEQL+4.62 shows that an increase in pH results in a lower Cd^{2+} in the cysteine-buffered medium than in the NTA-buffered one, which could well explain the observed difference in Cd uptake rates. Taking the results together, we speculate that the pH and the chemical speciation of Cd in the boundary layer of the alga are different from the conditions in the bulk solution, and that the chemistry of the boundary layer is influenced by the algal nitrogen source. Direct measurement of the pH in the boundary microenvironment could help elucidate the relative importance of biologically-mediated surface processes and abiotic chemical processes in determining metal bioavailability.

TP246 Effects of the nanoparticle size of ZnO on bacterial bioluminescence, seed germination, algal growth, and gene mutation

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The potential effects of different particle sizes of ZnO nanoparticles (NPs) were evaluated in the context of bacterial bioluminescence, seed germination, gene mutation in bacteria, and algal growth. Different sensitivities (EC_{50s}) were observed, showing the following orders of toxicity: bacterial bioluminescence (0.31–0.40 mg/L) > algal growth (1.08–2.90 mg/L) > seed germination (6.56–9.98 mg/L). The mutation ratios of the 5 and 15 nm NPs were 2.4 and 2.03, respectively, whereas they were 1.7 and 1.1 for 25 and 80 nm, respectively, at 100 mg/L ZnO. The effects of the particle size of ZnO differed according to the tested assays. Under exposure conditions with large sizes, the toxicity of ZnO NPs increased on seed germination, but decreased on algal growth and gene mutation. The toxicity results (EC_{50s}) of algal growth and seed germination showed statistically significant differences (p-values < 0.05) between 5 nm and 80 nm sizes. In the case of bacterial bioluminescence, no significant differences were observed among each of the sizes tested. These findings show that a toxicity evaluation of NPs needs to consider the different effects with respect to the particle sizes and tested organisms for an accurate assessment of the NP toxicity in ecosystems.

TP247 Effects of Cadmium and Nickel on cell cycle progression, growth and antioxidant enzymes of green algae Chlamydomonas reinhardtii

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Among aquatic organism, microalgae have an important role in aquatic system as they are a key component of food chains. It is crucial to has early assessment tools to evaluate effects of metals at the cellular level, which can lead to disturbance in structure and productivity of the algae community. Cd and Ni were evaluated on cell cycle progression, growth and antioxidant enzymes kinetic of the green algae C. reinhardtii. Synchronized cultures of this multiple fission dividing algae were used for the study. Aliquot from growing cultures were taken hourly during 36 hours. The attainment of commitment points (CP) was evaluated by transfering hourly aliquot into aerated tubes at 30 °C in the dark. In hourly samples analysis of cellular division, nuclear divison changes in cell size, protein and antioxidant enzymes activities of catalase, guaiacol peroxidase, ascorbate peroxidas, glutathion reductase were performed. The proportion of mother cells and daughter cells were assessed at the end of the cell cycle. Ecotoxicity of both metals was assessed by algal growth inhibition test, estimating toxicity endpoints, growth rates and concentration of chlorophyll a, chlorophyll b and carotenoids at the end of 96 hs of exposition. Both metals provoked a block of cell cycle at the highest concentration tested. At lower concentrations, cell cycle progression was observed with different pattern of attained CP, depending the exposure concentration. Antioxidant enzyme activities were inhibited at concentration above 0,05-1 mg/L (Cd, Ni). Cd affected both growth rates and pigments in a worse manner than Ni. The effects of both metals on concentration of pigments were less evident than the effects on growth rates, indicating a lower sensitivity of these parameters. Cd and Ni provoked severe damage on algal cell growth, cell cycle progression, photosynthetic pigments as well as modification of antioxidant enzymes activities. An integrated analysis is done discussing the consequences on population performance in natural environment affected by metal discharged from different anthropogenic sources.

TP248 Assimilation of contaminants by invertebrates after a coal ash spill

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Dietary exposure is an important pathway for metal uptake in aquatic organisms, and assimilation efficiencies of metals are often the most relevant parameter to quantify the bioaccumulation of metals in aquatic food chains. In field studies, however, metal body burdens in aquatic animals are often measured even though body burdens typically include gut content in the analysis. In benthic invertebrates collected from contaminated sites, gut contents can often contain sediments which can comprise a large proportion of the animal's body weight and can have elevated metal concentrations. While body burdens measured in this way can be useful in assessing exposure, risk can potentially be overestimated for metals that are not efficiently assimilated. Here we evaluated metal assimilation and bioconcentration in two invertebrate species with different feeding habits, the mayfly nymph (Hexagenia bilineata) and the silty horn snail (Pleurocera canaliculatum) at the site of the Tennessee Valley Authority's coal ash spill in Kingston, TN. We collected water, sediment, periphyton, and biota from sites both up- and down-stream of the coal ash spill and allowed a subset of animals to purge their gut contents before analysis to compare assimilation between metals, animals, and sites. Samples were analyzed for six elements which include both essential (selenium (Se), iron (Fe), zinc (Zn), and copper(Cu)) and non-essential (arsenic (As), mercury (Hg)) metals. We found that concentrations of the coal ash associated contaminants Se and As were higher in snails and nymphs collected from sites downstream of the ash spill than upstream. Spatial trends for Cu, Fe, and Zn did not indicate that exposure to ash affected bioaccumulation of these essential elements in either snails or nymphs. Selenium concentrations were higher in both snails and nymphs than in periphyton or sediment samples, but there were no significant differences between concentrations in purged vs. non-purged animals, suggesting high assimilation of this element. Arsenic, Fe, and Hg concentrations were higher in non-purged nymphs than in purged nymphs suggesting sediment concentrations of these elements were elevated with respect to their assimilation. Results from this study provide valuable information on the bioaccumulation of coal ash contaminants by invertebrates, which is relevant to evaluating risk at contaminated sites.

TP249 Acute and chronic ecotoxicity of safe by design copper oxide nanoparticles on the pond snail Lymnaea stagnalis

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Nanotechnology is a rapidly developing field in the 21st century, and the commercial use of nanomaterials for novel applications is increasing exponentially. Copper oxide nanoparticles (CuO NPs) are frequently employed for their antimicrobial properties in antifouling paints and other applications. Their extensive use can contaminate aquatic ecosystems. The main objective of this study was to evaluate and compare the aquatic toxicity of different CuO NPs through acute and chronic toxicity tests with different life stages of the snail Lymnaea stagnalis, a representative organism of the benthic ecosystem. Acute waterborne exposure was focused on the evaluation of the acute lethal toxicity of CuO NPs to juveniles (7-9 day old) of the pond snail L. stagnalis exposed for 96h at 20°C to Cu in a static experiment, either as pristine CuO NPs or "safe by design" CuO NPs. The latter were CuO-ASC and CuO-PVP NPs with respectively Na ascorbate and Polyvinylpyrrolidone as capping agents. LC50 value estimated in tests showed that CuO-ASC ($LC20_{96h}$ = 1540 $\mu g\ L^{-1}$ Cu) is the least toxic among all the CuO investigated. Chronic toxicity tests aimed to investigate the effects of exposure to CuO NPs pristine and "safe by design" on the reproduction to L. stagnalis. Young adult snails(22±2mm) were exposed to Cu as CuO NPs at 20°C for 30 days in a semi-static experiment. Endpoints such as: mortality, feeding rate and weight changes were also evaluated, along with the reproduction parameters. $LC50_{30d}$ value estimated in tests with pristine CuO NPs was 500 $\mu g\ L^{-1}$ Cu, indicating a 5 time fold higher toxicity than the acute test. Additionally, exposure to "safe by design" CuO NPs showed significant effects ($p< 0.001$) on the growth and reproduction parameters relative to the control; indicating, in contrast with acute exposure, an higher toxicity of CuO-ASC in the long term. The experiments' results demonstrate a time-related increasing toxicity of CuO NPs on L. stagnalis, emphasizing the need for more chronic study to accurately evaluate the impact of nanomaterials in the real environment. Furthermore, long-term experiment using juveniles L. stagnalis exposed to CuO NPs are ongoing, evaluating growth and time-related expression profiles of antioxidant enzymes and heat shock proteins response in snails to thermal shock. This research project is funded by the European FP7 project SUN "Sustainable Nanotechnologies".

TP250 Effects of Arsenic, Cadmium, Copper and Zinc on the Larval Survival of an intertidal gastropod, Nassarius festivus

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There are many kinds of industrial complexes and large cities in coastal areas of Korean peninsula. Benthic communities adjacent to these areas are subjected to environmental contamination by various kinds of anthropogenic toxicants including metals and persistent organic pollutants. Nassa snail, Nassarius festivus (Powys, 1835) is a small gastropod and one of dominant scavenger in benthic community inhabiting sandy and muddy intertidal and subtidal areas of western and southern coasts of Korea. Although there are studies on the effects of some metals on the acute and chronic responses of N. festivus, information on the sensitivity of larvae of this species is still lacking. The tolerance range to pollutants at larval stage can be a critical factor for the successful recruitment to adult population. To this end, we set the purpose of this study to know the acute responses of veliger larvae of N. festivus to 4 priority metals (As, Cd, Cu, and Zn). The 96-hr LC50 was 2.61 mg/L for As, 2.51 mg/L for Cd, 0.049 mg/L for Cu and 2.25 mg/L for Zn. The acute toxicity of Cu to veliger larvae of N. festivus was highest than other 3 metals. For As, Cd, and Zn, larvae of N. festivus was more sensitive than or comparable to Aplexa hypnorum (gastropod) and Tigriopus japonicus (copepod), less sensitive than Neomysis japonica (mysid) and larvae of Mytilus gallo-provincialis (bivalve). However, for Cu, N. festivus was more sensitivity than any other species of taxonomic groups (copepod, mysid, amphipod, isopod, bivalve, fish etc.). We concluded that the bioassay using this species can be quite useful for the evaluations of environmental quality and risk assessments of contaminated marine ecosystems.

TP251 Effects of diet on embryogenesis of the great pond snail, Lymnaea stagnalis exposed to cadmium, tributyltin or pyraclostrobin

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Diet quality can have a strong impact on organismal fitness although diet quality is infrequently considered as a factor in toxicity tests, especially in embryotoxicity testing. The purpose of this study was to evaluate how diets of adults that differ in nutritional content affect sensitivity to cadmium, tributyltin or pyraclostrobin in Lymnaea stagnalis offspring. Three groups of hatchlings were fed a different diet each, either lettuce, high-caloric pellets, or a combination of both alternating every week until they reached reproductive maturity. Snails fed pellets and both diets had similar growth in length and mass; however, snails fed lettuce showed lower growth until about the 4th month when all groups had the same length and mass. Egg masses were collected from 6-7 month old adult snails fed each diet and exposed to three concentrations of either cadmium, tributyltin or pyraclostrobin. We assessed time to hatch, hatching success and the different developmental stages of the embryo. Results indicated that for the three contaminants egg masses from parents fed lettuce hatched earlier than egg masses from parents fed both diets. For cadmium and pyraclostrobin, egg masses from parents fed pellets did not hatch at all, not even the controls. Interestingly, control egg masses from parents fed pellets and exposed to tributyltin did not hatch either but a few individuals from the lowest concentration did. The nutrient rich pellet diet was surprisingly inferior to lettuce and apparently altered the biology of the study organisms causing offspring to be non-viable. These observations on offspring performance suggest that there are important diet effects that can strongly influence responses but in a non-intuitive way. Additional studies are needed to better understand the mechanisms by which increased nutrient availability affects an organism's offspring when exposed to a toxicant.

TP252 Toxic and genotoxic effect of cadmium, chromium and lead on Japanese oyster Crassostrea gigas larvae

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The Japanese oyster is an introduced species that grows in the Mexican Pacific coastal systems, because of its economic importance we performed an evaluation of the genotoxic effects of the metals: Cd, Cr and Pb on Japanase oyster larvae. Bioessays, lasting 72 hours, were carried out with 5 concentrations of each metal and its mixture (1:1). Lethalconcentration 50 (LC_{50}) were determined in the assays, and surviving organisms was performed evaluation genetic damage by means of unicellular electrophoresis technique (comet assay) in a tissue sample, which were revised between 300 to 500 cells, evaluating the frequency of occurrence of cells with damage (tails) and its length. The toxicity of metals based on the LC_{50} calculated was (high to low effect): Cd > Cr > Pb. The most toxic mixture of metals was Cd + Cr and less toxic Cd + Pb. The interaction in the mixtures of metals, Cd + Cr and Cd + Cr + Pb was potentiation, with magnification factors from 5 to 2 respectively. The Kruscal-Wallis test indicated that significant differences exist between the level of DNA damage in organisms exposed to different metals and the control. The metal with major genotoxic effect was cadmium followed by chromium. Lead showed the lowest genotoxic effect. The mixture of metals with more genotoxic effect was Cd + Cr and the mixture of the three metals. Metal concentrations used in this study have been reported in sediments from coastal areas in the state of BC and BCS. for this reason is important to continue research and monitoring to detect responses that indicate possible damage to oyster populations by the action of different tensors, to prevent irreversible deterioration of the stocks in the medium and long term.

TP253 Short-term induced molecular stress responses in coelomocytes of Eisenia fetida earthworms in vivo exposed to silver nanoparticles

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In recent years the production of a great variety of products containing nanoparticles (NPs) has increased massively. The subsequent release of NPs into the environment has created a need to assess the potential ecological risk in soil, water and air. Silver nanoparticles (Ag-NPs) have the highest degree of commercialization due to their high thermal and electric conductivity, high catalytic activity, and powerful antimicrobial properties. Eisenia fetida is a model specie in soil toxicity studies and has been broadly used due to its sensitivity to different toxicants at different levels of biological organization. The main aim of the present investigation was to understand the effects produced by AgNPs (5.08±2 nm sized and PVP-PEI coated) in comparison with the soluble form of the metal ($AgNO_3$) at molecular level in coelomocytes of E. fetida at different exposure times. E. fetida were in vivo exposed to different concentrations of Ag-NPs and $AgNO_3$ (0.05 and 50 mg Ag/kg soil) through OECD artificial soil for 1, 3 and 14 d. Then, the transcription levels of selected genes associated to oxidative stress (Catalase) and metal detoxification (MTs-metallothioneins) were determined in coelomocytes extruded from exposed earthworms. In addition, the enzymatic activity (Catalase) and protein content (MTs) were quantified. The responses varied significantly among days, exposure concentration and Ag form. Exposure to Ag-NPs led to a significant induction of CAT at day 1, followed by an increase in its transcription levels after 3 and 14 d of exposure. Similarly, exposure to $AgNO_3$ induced the transcription of CAT at day 1 but at day 14 a down-regulation was observed. The CAT activity increased at both treatment and exposure times (1 and 3 d). After 14 d of exposure, CAT activity was inhibited at the highest concentration tested. The highest increase of MTs at protein level was observed after 3 d of exposure. Our results indicate that short-term exposures to Ag-NPs induced early molecular stress responses (MT induction and oxidative stress) in coelomocytes that precede other responses at higher levels of biological organization. The responses in translational level in E. fetida tissues were according. The study indicates the importance of using integrative biomarkers for the evaluation of the potential risk of Ag-NPs in soils.

TP254 The effect of sediment pH on the toxicity of silver nanoparticles to Lumbriculus variegatus

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The toxicity of silver nanoparticles (AgNP) to aquatic organisms is a major concern, as the increasing use in industry and consumer products leads to increasing release of AgNP into the aquatic environment. Sediments are believed to be a major sink for AgNP, but only limited amount of data is available on the toxicity and behavior of sediment-associated AgNP. We studied the effects of sediment pH on the toxicity of AgNP on benthic oligochaeta Lumbriculus variegatus. In addition, the toxic effects were related to the bioaccessible amount of dissolved Ag compounds in the sediment. Artificially prepared sediments (pH 5 and pH 7) were spiked with uncoated AgNP (7-1365 mg/kg sediment dry height), polyvinylpyrrolidone coated AgNP (9-1132 mg/kg sediment dry weight) and silver nitrate ($AgNO_3$, 4-334 mg/kg sediment dry weight) as dissolved Ag reference. Reproduction, mortality and inhibition of growth were used as endpoints for the 23-day toxicity test. The concentration of dissolved Ag in sediment was assessed by two-step extraction procedure followed by centrifugation to remove AgNP from the extracts. The concentration of dissolved Ag in the AgNP-spiked pH 7 sediment was negligible, and no toxicity was observed even in the highest (1365 mg of Ag/kg dry sediment) exposure concentration. In pH 5 sediment, the toxicity of both AgNP was significantly promoted, and toxic effects were observed already at the concentration of 38 mg Ag/kg sediment dry weight. When the effects were plotted against the concentration of bioaccessible dissolved Ag in the sediment, $AgNO_3$ treatments resulted in toxicity at the similar level as AgNP treated pH 5 sediment. Our results indicate that the dissolution of AgNP is significantly higher in the sediment with low pH. Also the toxicity of AgNP and $AgNO_3$ to L. variegatus was well related to the concentration of dissolved Ag in the bioaccessible fraction of the sediment.

TP255 Water level management effects on Cd, V, and Zn biogeochemistry and toxicity to Hyalella azteca and Daphnia magna

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Current understanding of the fate and effects of vanadium (V) on aquatic organisms is limited, with relatively few investigations of sediment dynamics. Chronic thresholds for toxicity have been established for dissolved V in water, but no equivalent exists for sediment and very few studies have considered V speciation and toxicity. Further, binding of V to sediment ligands, such as iron/manganese oxy-hydroxides, sulfides, and carbonates is likely to decrease V toxicity in field settings. The objective of this study is to assess the role of hydrologic manipulation on the speciation and toxicity of V to benthic and water column organisms. Sediments were collected from a dammed-river reservoir located in a former mining area, and these sediments contained elevated concentrations of vanadium (range: 635 to 1620 $\mu g\ g^{-1}$) and other metals. Water level fluctuation experiments were conducted to emulate 7-day drying and re-inundation periods. Porewater and sediment metals (Ca, Cd, Cu, Fe, Mg, Mn, Ni, V, and Zn) and important metal binding phases (iron-oxide speciation, acid-volatile sulfide) were quantified. Test organisms H. azteca and D. magna were exposed to sediments during the first 7 and 10 days, respectively, of re-inundation to assess toxicity. Preliminary results show V to be highly bound to sediments and generally not biologically available. Surface water V (+5) remained low in concentration throughout the experiment (< 17 $\mu g\ L^{-1}$), below reported thresholds for surface water toxicity. While a small flux of V (+5) was released into porewater upon re-inundation of oxidized sediments, the concentration also remained low (0.2 – 6.5 $\mu g\ L^{-1}$). Upon inundation, a flux of Zn and Cd into porewater and surface water exceeding the USEPA threshold for chronic and acute toxicity to freshwater organisms was observed for two of the three sediments tested. This increase in Zn and Cd is possibly tied to decreased survival observed for H. azteca during the experiment. Results from this study suggest that V bioavailability and environmental risk is low despite relatively high sediment concentrations, and V is less susceptible to redox changes associated with water level fluctuations than other more labile metals.

TP256 A comparison of the effect of inorganic chemicals to Daphnia magna

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Various inorganic chemicals exist in environment, their come from not only natural source but also artificial source. Inorganic chemicals include metal, metal ion, metal salts, and nano materials. We expect that the metal salts also affect organisms (for example the metal ion in aqueous solution). The following studies mainly use metal salts as inorganic chemicals. Since metal chloride, metal nitrate and metal sulfate is one of the forms which are stabilized as for a metal salt. Chloride is one of the most popular atomic element in the sea. Nitrate salts play important role in nitrogen cycle. Sulfuric acid is one of the most important reagent in industrial chemistry and good applications for oil refining, wastewater processing, and mineral extraction. And sulfate salts play important role in sulfur cycle. Median effective concentration (EC_{50}) were selected as prediction of adverse effect of Daphnia magna. The EC_{50}s were used from previous studies and several literatures. D. magna immobilization test is listed in OECD Guidelines for the Testing of Chemicals (TG202). Acute EC_{50}s of alkali metal salts employed values of metal chloride, metal nitrate and metal sulfate. Following listed compounds. Lithium chloride (LiCl), sodium chloride (NaCl), potassium chloride (KCl) rubidium chloride (RbCl) and Cesium chloride (CsCl) were used for metal chloride. Lithium nitrate ($LiNO_3$), sodium nitrate ($NaNO_3$), potassium nitrate (KNO_3), rubidium nitrate ($RbNO_3$) and Cesium nitrate ($CsNO_3$) were used for metal nitrate. Lithium sulfate (Li_2SO_4), sodium sulfate (Na_2SO_4), potassium sulfate (K_2SO_4), rubidium sulfate (Rb_2SO_4) and Cesium sulfate (Cs_2SO_4) were used for metal sulfate. In acute EC_{50}s of alkali metal salts have poor correlation with ionic radius, covalent radius aqua ion diameter and electronegativity (r = -0.4 – 0.4), on the other hand, have good correlation with abundance in earth's crust, sea and space (r = 0.7 – 0.9). These results suggest this organism historically play important role in aquatic ecosystem.

TP257 Assessment of the Effects of Mining-Derived Metals on Caged Crayfish and Wild Crayfish Populations in Madison County, Missouri, USA

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Deposits of lead (Pb) and other metals of southeastern Missouri, USA have been exploited since the 1700s, which has left a legacy of metals-contaminated streams. Contamination of fish and other aquatic biota, alteration of fish and invertebrate communities, and public health advisories against human consumption of Pb-contaminated fish have resulted. Previous studies in mining districts in Missouri have documented adverse effects on crayfish populations in mining-contaminated streams, but streams in mine-impacted Madison County, Missouri have not been previously assessed. Crayfish population density surveys and in-situ toxicity tests with caged laboratory-reared crayfish (Orconectes quadruncus) were conducted at sites upstream and downstream of historical mining areas in the Little Saint Francis River watershed. O. quadruncus, an endemic species in this watershed, is listed as imperiled by the state of Missouri and has been petitioned for listing as a Federal endangered species. Sites downstream of mining areas had significantly higher metal concentrations in water, sediment, organic matter and biota, and significantly lower riffle densities of Orconectes luteus and O. quadruncus compared to reference sites. Survival of caged O. quadruncus was not significantly different at sites upstream or downstream of mining-impacted sites. Nickel (Ni) and cobalt (Co) concentrations were elevated in water, and Ni, Co, and copper concentrations were elevated in crayfish at sites downstream of mining. Probable effects concentration quotients for metals in fine (< 250 µm) sediment, which were primarily driven by Ni and Pb concentrations, indicate that sediments are likely toxic to sediment-dwelling organisms at all sites downstream of mining areas. Crayfish are important prey for many aquatic and terrestrial animals and they play important roles in organic matter processing and nutrient cycling in stream food webs. Thus, adverse effects of metals on crayfish populations will likely extend to other components of Ozark streams and surrounding ecosystems.

TP258 Concentrations of heavy metals in tissues of commercially-harvested red crabs from the northwestern Atlantic

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Red crabs (Chaceon quinquedens) are benthic and inhabit the continental slope of the Atlantic, Pacific and Indian oceans, usually at depths of 200 to 1800+ meters. There is a small commercial, federally-managed fishery on the US Atlantic coast for deep-sea red crabs. However, management is hindered by the absence of information on their biology, abundance, growth, age, reproduction, or contaminant concentrations. Red crab samples were collected from three locations from the northwestern Atlantic, and metal concentrations were determined in edible muscle and hepatopancreas. Metal concentrations (As, Pb, Hg, and Cd) did not differ among locations or sexes, and concentrations were not correlated with carapace width or length. Median As, Cd and Pb concentrations in muscle were 62, 0.35, and 0.35 mg/kg dry weight, respectively. Most Hg in muscle was methyl mercury, but inorganic mercury was dominant in hepatopancreas. Median Hg concentration was higher in edible muscle (1.07 mg/kg dry weight, range 0.22-4.46) than in hepatopancreas (0.65 mg/kg, range 0.18-2.4). Most Hg in muscle was methyl mercury, but inorganic mercury was dominant in hepatopancreas. Some crabs exceed established consumption advisories for Hg in seafood.

TP259 The effect of temperature on ecotoxicity of nano copper oxide to midge species

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Temperature is one of the most important factors changing toxicity to organisms in ecosystem. In addition, we are living in the era of rapid climate change. There are few studies about nano toxicity related to temperature in mesocosm. This study was about the temperature-dependent toxicity of nano copper oxide to midge species, Chironomus yoshimatsui, in microcosm consisting of artificial sediment and water. We investigated and compared the protein patterns of C. yoshimatsui exposed to different temperature and nano concentrations using SELDI-TOF MS (surface enhanced laser desorption/ionization time-of-flight mass spectrometry). The low level of nano copper oxide didn't effect on lethality of tested organisms, but there was alterations of protein pattern with temperature. This suggests that we need an in-depth study about nano toxicity with temperature.

TP260 Trace Element Accumulation in Six Dragonfly Nymph Genera from a Wetland Constructed for Cu and Zn Effluent Treatment

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Dragonfly adults and their aquatic immature stages are an important part of food webs and provide a link between aquatic and terrestrial components. Our previous work in a stream receiving coal combustion waste indicated dragonfly nymphs accumulate coal-ash contaminants such as Cu and Zn. The H-02 wetland system was constructed on the Department of Energy's Savannah River Site, Aiken SC to treat building process and stormwater runoff water from a portion of H-area. Constructed wetlands play an important role in the SRS environmental plan to achieve both federal and state regulatory compliance for the discharge of effluent waters. The H-02 wetlands were built to remove trace metals, primarily Cu and Zn, from the water before release to state waters. The system consists of a retention basin, two wetland treatment cells, an effluent pool, and a discharge stream. We are using 6 genera of dragonfly nymphs as biomonitors to assess bioavailable contaminants throughout all components of the wetland system as well as two reference wetlands. A total of 600 composite samples were formed by pooling individuals within size classes developed from length frequency analysis. Whole body concentrations of 15 elements (Cu, Zn, Pb, B, Al, V, Cr, Fe, Mn, Co, Ni, As, Se, Cd, Ba) were determined for each composite. Patterns of accumulation in dragonfly nymphs varied by genus and element. Variation among genera clearly exceeded variation within genera indicating that genus is a reasonable taxonomic level for both spatial and taxonomic comparisons. Some elements such as Cu were elevated above reference site levels throughout the wetland system and not lower in the effluent pool. This suggests significant escapement of contaminants through the system. Whether Mn accumulated to higher levels in the upper or lower end of the system depended upon genus. Some genera accumulated Cd to higher levels in the treatment cells, but to only low levels. Zn appears to be better regulated by the nymphs and some elements accumulated to only low levels. Anax and Tramea that typically inhabit vegetation well above the substrate often accumulated lowest concentrations of many elements. In contrast, Erythemis, Libellula and Pachydiplax, genera that more frequently inhabit a zone closer to the substrate, tended to accumulate the highest concentrations. Association with the sediment may increase accumulation of some elements in dragonfly nymphs.

TP261 Assessing the trophic transfer of selenium to Hyalella azteca and Pimephales promelas through a diet of field-collected periphyton communities

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The contamination of aquatic systems with selenium (Se) is of significant global concern. A variety of natural and anthropogenic sources of selenium exist, and their source can contribute different forms and concentrations of Se (eg. selenate [SeO_4?^{2}] and selenite [SeO_3?^{2}]) to aquatic environments. Inorganic forms of Se are assimilated and bioaccumulated by aquatic microorganisms and biotransformed into organoselenium compounds. Organic-Se is then transferred to higher trophic levels via dietary pathways. The present study aims to quantify the trophic transfer factors of selenomethionine to a primary consumer through the biotransformation of inorganic oxyanion Se forms (selenate and selenite) by field-collected communities of periphytic microorganisms. Biofilm samplers will be placed in the photic zone of uncontaminated lakes in northern Saskatchewan, Canada, and allowed to accumulate natural communities of microorganisms representative of these lake habitats. Samples will be collected from each biofilm sample for microscopic and metagenomic characterization of the biofilm communities. Samples will then be exposed in the laboratory to aqueous concentrations of selenite and selenate respectively, and Se uptake by the biofilm will be quantified. The amphipod Hyalella azteca, a primary consumer characteristic of Canadian freshwater ecosystems, will graze on the selenized biofilm communities to determine trophic transfer efficiencies as a function of microorganism community structure. Selenized amphipods (batch cultured) will be fed to fathead minnows (Pimephales promelas) to further characterize Se trophic transfer in this representative aquatic food web. This research will serve to assist in improving environmental risk assessment strategies for the release of Se into aquatic environments.

TP262 Effects of copper on primary culture of a rainbow trout gill epithelium (DSI system): intracellular and paracellular aspects

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The gill is a multifunctional organ in fish and the critical site of acute toxicity to waterborne contaminants, including copper. Alternative approaches are now needed in fish toxicology testing due to pressures for refining, reducing and replacing (3Rs) live animal studies. A promising alternative method is the primary culture of a freshwater rainbow trout (Oncorhynchus mykiss) gill epithelium on a flat permeable membrane (DSI system). This system is unique in its ability to generate a polarized epithelium, which can tolerate prolonged freshwater exposure at the apical surface. Thus, the present study investigated the toxicological effects of copper on gill epithelial cultures of rainbow trout (DSI), emphasizing intracellular and paracellular aspects. The primary gill epithelia were prepared using the double seeding technique and grown at 18°C in culture media. The development of the intact gill epithelium was monitored daily through measurements of transepithelial electrical resistance (TER). When the TER achieved a plateau (7-8 days), a heterogeneous gill epithelium similar to that found in vivo was completely formed. At this point, the exposure of the gill epithelium was initiated by replacing cell culture media on the apical side with filtered freshwater. The gill cell cultures were exposed to 3.14µM and 15.75µM of copper for 24h. In order to evaluate the effects of copper, the parameters analyzed were: copper accumulation, cellular ionic content (sodium, calcium, magnesium, potassium and chloride), carbonic anhydrase and Na,K-ATPase activities and gene expression, paracellular permeability (PEG) and tight junction protein analyses. TER values differed between control and exposed groups until 6 h of exposure, after which time the values remained without significant difference. (IDRC, NSERC Discovery).

TP263 New insights into the toxic effects of arsenic with cellular evidences in the green algae and zebra fish

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Arsenate and arsenite are the primary chemical forms of arsenic to exert toxic effects on biota. The aim of our study was to explore the use of several novel biomarkers and laboratory tools for indicating the response of green algae and zebra fish exposure to arsenic in the aquatic environment. Two lines of new evidence are included as follows: (1)Arsenate exposure caused subcellular damage to chloroplast and decreased PSIIefficiency in green algae. The EC50 values of growth inhibition to C. pyrenoidosa and S. Quadricauda were 5.14 $mM{\cdot}L^{-1}$ and 1.06 $mM{\cdot}L^{-1}$, respectively. TEM images showed the structural damage of chloroplast, including the vacant thylakoid membrane, an increased number of starch grains and diminished pyrenoid following 24-h exposure of arsenate. The maximum relative electron transport rate (ETRmax), initial slope of rapid light curve (α) and half saturation light intensity (Ik) were significantly reduced and the reduction was positively correlated with the concentration of arsenate. ETRmax, α, and Ik were decreased. The effects of arsenate on the maximum relative electron transport rate, light utilization efficiency, and strong light tolerance of S. quadricauda were more significant than those of C. pyrenoidosa. (2) Arsenite induced disturbance on acid-base and electrolyte balance distinct from the normal zebrafish embryos were demonstrated. The dose-dependent activation of immunoregulatory NF-κB signaling pathway was revealed with ion fluorescent probes and the dual-luciferase reporter system. The results showed that arsenite at 0.5-5mM significantly inhibited the survival and hatching rates of zebra fish. The calculated 96-h LC50 was 1.94 mM and the hormesis of arsenate was observed on hatching rate. Furthermore, intracelluar pH and Ca^{2+} concentration of zebrafish embryos decreased significantly after treatment with sublethal concentrations of arsenite (0.5~ 2.0 mM). Under this exposure condition, arsenite exhibits a significant time-dependent activation of NF-κB signaling pathway. The results indicated that arsenite induced disturbances of acid-base and electrolyte balance might be the underlying cause for the activation of NF-κB signaling pathway. This work contributes to the better understanding of the chemical regulation mechanism of arsenic toward aquatic immunotoxicity. Our results will help differentiate the toxic biota effects of arsenic from other co-existed contaminants from arsenic-based insecticides and metal processing wastes.

TP264 Water chemistry alters toxicity of copper oxide nanoparticles in early life-stage zebrafish

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The increased use and global spread of nanoparticle- (NP-) enabled technologies means that NPs are expected to be released into diverse aquatic environments. Characterising how variable abiotic factors such as water pH may alter NP toxicity is therefore a priority for risk assessment. Furthermore, NPs are unlikely to enter the environment as continuous discharge streams and pulsed intermittent exposures are more ecologically relevant to study toxic effects in organisms. The aims of this study were to investigate how pH and other water chemistry variables altered the toxicity of copper oxide (CuO) NPs in early life stage zebrafish and also to consider toxicity of CuO NPs in a pulsed exposure experimental design. Data showed a significant stepwise decrease in toxicity of CuO NPs at pH 5, 6 and 7 which was different to what was observed for Cu^{2+} (as $CuSO_4$). Calculated 96 h LC_{50} at pH 5, 6 and 7 were: 4.7, 24.8 and 150.8 mg L^{-1}, respectively. Calculation of NP dissolution at these concentrations also indicated that dissolution decreased with increasing pH and was in broad agreement with the corresponding LC_{50} of Cu^{2+}. This suggested a central role of Cu^{2+} dissolution in CuO NP toxicity. This observation was further confirmed in exposures to CuO NPs and Cu^{2+} at corresponding LC_{10} values. Both CuO NPs and Cu^{2+} caused same magnitude depletions in whole zebrafish glutathione concentrations and inhibitions of Na^+/K^+-ATPase activity. Accumulation of Cu in tissues was also measured. Further experiments exposed zebrafish embryos to CuO NPs in discrete windows to assess sublethal and lasting effects at 96 h of development from early (0-24 h), pulsed intermittent (24-48, 72-96 h) and continuous (0-96 h) exposures. This study is part of the Sustainable Nanotechnologies Project funded by the EU 7th Framework Programme.

TP265 Tissue Distribution of Essential and Nonessential Trace Elements in Ocean Sunfish (Mola mola) and Sharptail Mola (Masturus lanceolatus)

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Sharptail mola (Mola lanceolata) and ocean sunfish (Mola mola) stranded on the Atlantic coast of Florida, USA during 2010 and 2011 (with an additional stranding of sharptail mola during 2014). These two species are not commonly observed within Florida waters and the multiple mortalities during these time periods were atypical. During each stranding event we collected fish length, sex and multiple tissues (muscle, gill, liver, kidney, spleen, gonad and heart) which were analyzed for the concentration of seven essential trace elements (Co, Cu, Fe, Mn, Ni, Se, Zn) and seven nonessential trace elements (Ag, As, Cd, Cr, Hg, Pb, Sn) using Inductively Coupled Plasma Mass Spectrometry (ICP-MS). With the exception of Ag, Hg, Pb and Sn, all elements were detected in all tissues of both species. Tin was below detection in all samples and Ag, Hg, and Pb was not detected in all tissues. Of all the investigated essential trace elements, Fe was found in highest concentration in the liver, kidney, spleen and gonad, whereas Zn was highest in the muscle, gills and heart. The average concentration of Fe in the liver was 8.8-times higher in the sharptail mola than in ocean sunfish (2110 µg/g dry wt and 239 µg/g dry wt, respectively). For the nonessential elements, As was found at the highest concentration in all investigated tissues. Mercury was found at low concentration in the muscle tissue of all fish, averaging 0.58 µg/g dry wt for sharptail mola and 0.17 µg/g dry wt for ocean sunfish, reflecting the low trophic position of both species. The average concentration of Cd in the liver was 4 times higher in sharptail mola than in ocean sunfish (8.17 µg/g dry wt and 2.11 µg/g dry wt, respectively); this concentration is elevated compared to other fish species, but below the 10 µg/g wet wt concentration that is known to cause deleterious effects in the liver. This is the first study to examine a full suite of trace elements in multiple tissues from two globally occurring mola species and the first to analyze any contaminant type in sharptail mola.

TP266 Subcellular distribution of trace elements in the livers of Alaskan yelloweye rockfish

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Yelloweye rockfish (Sebastes rubberimus) are an extremely long-lived species (up to ~120 years), which inhabit the coastal waters of Alaska. Due to their long lifespan, yelloweye are known to accumulate high levels of mercury, and potentially other trace elements, in their tissues. Relatively little is known about the subcellular distribution of trace elements in the organs of yelloweye rockfish, which can provide important insight into detoxification/toxicity mechanisms at the subcellular level. To address this question, we collected yelloweye rockfish (n=8) from the eastern coast of Prince of Wales Island, Alaska in 2014. We determined the subcellular partitioning of trace elements (total Hg, Cd, Pb, and As) in yelloweye liver by carrying out a partitioning procedure to separate liver cells into putative metal-sensitive fractions (cytosolic enzymes, organelles) and detoxified metal fractions (metallothionein-like proteins and peptides, granule-like structures) using differential centrifugation, NaOH digestion and heat denaturation techniques. The resulting fractions were then analyzed for Hg with a direct mercury analyzer and for trace element concentrations by ICP-MS. For Cd, Pb, and As, the greatest contributions were found in the detoxified fractions, whereas the majority of total Hg was found in sensitive fractions. Results indicate that although yelloweye sequestered and immobilized potentially toxic trace elements, detoxification did not occur to the same extent for all elements (Cd > As > Pb > Hg). In yelloweye rockfish livers, the accumulation of non-essential metals in sensitive sites could lead to deleterious effects at the subcellular level, which should be evaluated in future studies.

TP268 Cadmium potential as endocrine disruptor in catfish Rhamdia quelen

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Several studies demonstrated that cadmium (Cd) can impair the endocrine system in mammals, including humans and other vertebrates, as fish. The Cd effects in endocrine system can include alteration of hormones concentration, and in activity and/or in expression of enzymes, as aromatase. Cd can promote changes in integrity, development and maturation of gonads. The aim of this study was to evaluate the Cd effects in the reproductive system in males and females of the freshwater fish Rhamdia quelen. Fish were exposed (separated by gender) to different Cd concentrations (0, 0.1, 1, 10 and 100 µg L-1). The cadmium was replaced daily with 1/3 of water change. After 15 days of exposure, animals were anesthetized, the blood was sampled for estradiol and testosterone concentration measurement, and they were euthanized by medullar section and weighed. Gonads were weighed for gonadossomatic index (GSI) calculation, and used for genotoxic biomarker (comet assay) and biochemical biomarkers analysis: activities of catalase, glutathione peroxidase, superoxide dismutase, glutathione S-transferase, the concentration of glutathione reduced and levels of lipid peroxidation. Hypothalamus (HYP) expression of brain aromatase (CYP19a1b) was carried out by real time PCR. Normality of the data was evaluated by Kolmogorov Smirnov. Normal data were tested by one-way ANOVA, followed by Newman-Keuls and non normal data by Kruskal Wallis followed by Dunn with significance level of $p< 0.05$. No significant changes were observed in biochemical biomarkers, comet assay and GSI in gonads (testicles and ovaries) of exposed animals compared to control group. These results indicated that Cd did not cause stress oxidative, genotoxicity and cell membrane damage in gonads. Estradiol and testosterone concentrations and brain aromatase gene expression were not altered by Cd exposure. Thus, it was not detected endocrine disruptor effect of those Cd concentrations in the biomarkers tested for this catfish. One possible explanation is that the Cd, in the same conditions of this work, is accumulated mainly in the liver of R. quelen, indicating that the metal cannot reach the gonadal tissues in 15 days. Others studies demonstrated that longer exposures to Cd, as 30 or 60 days, cause toxic effects on fish gonads. Therefore, others parameters should be evaluated before conclude this investigation, since for some fish species is well documented effects of Cd in impairment of the endocrine system.

TP269 Evaluation of mercury concentrations in harvestable-sized fish from freshwater resacas in south Texas, USA

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The bioaccumulation and biomagnification of mercury (Hg) were investigated in freshwater fish from resacas in Brownsville, Texas. Resacas are a series of secondary channels (distributaries) and oxbow lakes left from flooding periods of the Rio Grande River. One of the few types of freshwater wetlands in extreme southern Texas, resacas provide valuable aquatic habitat and fishery resources. They are subjected to runoff pollution from urban centers and agricultural operations, creating potential health concerns for local subsistence fishers who use the resacas heavily. About 90 catchable size fish (20-70 cm total length) from eight species were collected from eight different resacas with known runoff pollution, and edible muscle was analyzed for total mercury. Total mercury was highest in freshwater drum (Aplodinotus grunniens, median 0.71 mg/kg dry weight) and alligator gar (Atractosteus spatula, median 0.77 mg/kg dry weight), and lowest in invasive armored catfish (Loricariidae) and tilapia (Cichlidae), median Hg 0.07-0.04 mg/kg dry weight, respectively. Stable isotope analyses are underway to relate Hg concentrations to trophic position and diet. Some fish may exceed risk-based consumption advisory limits, although there are presently no advisories for these waters. This study provides baseline data for mercury accumulation in fish from this area and for risk assessments for subsistence fishers and minority groups that regularly consume fish from resacas.

TP270 The effects of food web complexity on mercury bioaccumulation in upper trophic level fish

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Benthic macroinvertebrate communities are commonly monitored as indicators of water quality, and can be particularly useful in assessing changes in water quality due to contaminant exposure. Mercury (Hg) is a globally important contaminant which has recently been recognized as a concern in stream ecosystems. Mercury (especially when transformed to methylmercury (MeHg)) is one of the only metals known to biomagnify in aquatic food webs, leading to elevated concentrations of this toxicant in fish. Mercury is predominantly accumulated in fish through the food chain rather than aqueous exposure, so food web length and complexity play important roles in controlling Hg bioaccumulation in fish. Here we examined trends in invertebrate community structure and density at two sites over a 30 year time period in East Fork Poplar Creek (EFPC) in East Tennessee to determine if Hg bioaccumulation patterns in resident fish within this Hg-contaminated stream could be explained. We examined changes in overall invertebrate community structure (e.g., species richness and EPT richness) and temporal trends in different invertebrate feeding guilds. We measured Hg and MeHg concentrations in invertebrates in 2014-2015 to infer the magnitude of Hg trophic transfer from each invertebrate guild over time, comparing predictions to long term Hg bioaccumulation trends in fish. While benthic macroinvertebrate community surveys do not provide direct information on Hg bioaccumulation, long term changes in community structure provide useful information relevant to understanding food web dynamics that can affect trends in Hg bioaccumulation in upper-level consumers.

TP271 Acute toxicity of copper to the larval stage of three species of ambystomatid salamanders

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Copper (Cu) appears to be consistently toxic to amphibian species relative to other taxa. There has been limited Cu toxicity data for salamanders, and much of the data available used embryos. We performed acute toxicity experiments with three species of ambystomatid salamanders using the larval stage. We found very high toxicity for these three species compared to previously published research on the embryo stage. Specifically, the 4 d LC50s for Ambystoma tigrinum and Ambystoma opacum were 35.3 µg/L and 18.73 µg/L, respectively. The same test concentrations of Cu caused similar toxicity to Ambystoma talpoideum (LC50 = 47.88 µg/L), but exposures required up to 48 days to elicit the same toxicity. Our results suggest that Cu is much more toxic to larval salamander stages than for embryos, which has been found for anurans as well.

TP272 Concentration of copper in gulls: A Review of published data

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Gulls are birds that are present in most aquatic and terrestrial ecosystems. These birds are excellent bio-indicators of anthropogenic pollution, because they are opportunistic, omnivores and coexist with humans. They are particularly important to assess the ecosystem´s health because can reflect the environmental levels of pollutants. Copper is an essential trace element which can become toxic depending on dosage. We reviewed the Cu levels (dry weight) in different species of gulls, taking into account the biological matrix analyzed and the geographical location. The highest Cu concentrations were reported in Larus argentatus (19.87 ± 8.05 µg/g, n=18), whereas the lowest levels were in Larus genei (0.33 µg/g, n=18). The highest Cu concentrations were reported in excreta (60.1 µg/g, n=13), and the lowest in the subcutaneous fat (0.77 µg/g, n=5). With regard to the geographic location, the higher and lower Cu concentrations were reported in the Southern Hemisphere (11.41 ± 6.68 µg/g, n=166) and in the Northern Hemisphere (9.78 ± 8.61 µg/g, n = 1583), respectively. According to metal distribution in gull matrices, the Cu levels are as follows: excreta > liver > kidney > muscle > heart > feathers > stomach contents > intestine > brain > lung > salt gland > uropygial gland > gallbladder > gonads > stomach > pancreas > eyeball > eggs > skin > bones > blood > subcutaneous fat. High Cu levels in excreta may correspond to detoxification processes. Since most of the data at global scale are from Northern Hemisphere, future studies should be conducted in gulls from Southern Hemisphere. The gull excreta can be a good biological matrix for monitoring Cu. Standardized methodology for Cu detection in excreta is quite needed in order to compare data at global scale. Acknowledgements: Winfred E. Espejo is a scholarship CONICYT-Chile for PhD studies. This study was financially supported by FONDECYT-Chile 1140466 granted to R. Barra.

TP273 Concentration of Manganese in gulls: A global overview

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Gulls are present in extensive aquatic and terrestrial ecosystems. They are typically coastal species and fly long distances. They are excellent bio-indicators of pollution, because they are opportunistic, omnivores, and it is often possible to see them in towns, far to the coast. They are particularly important to assess the ecosystem´s health and can reflect the environmental levels of pollutants. In this work, we made an extensive review about gulls and manganese (Mn), to understand in a better way, this trace element distribution in the environment and its biological matrix selection, at a global scale. We reviewed the Mn levels (dry weight) in different species of gulls, depending on the biological matrix analyzed and the geographical location. This analysis gathered bibliographic data from Scopus, Springer, Web of Science and Science direct. The highest Mn concentrations were reported in Larus crassirostris (14.93 ± 32.5 µg/g, n=889), whereas the lowest levels were in Larus atricilla (0.003 ± 0.0017 µg/g, n=10). The highest Mn concentrations were reported in stomach contents (78.6 µg/g, n=20), and the lowest in the subcutaneous fat (0.18 µg/g, n=5). With regard to the geographic location, the higher and lower Mn concentrations were reported in the Southern Hemisphere (2.37 ± 4.08 µg/g, n= 61) and in the Northern Hemisphere (7.84 ± 21.62 µg/g, n = 2205), respectively. According to metal distribution in gull matrices, the Mn levels are as follows: stomach contents > feathers > liver > kidney > pancreas > gonads > salt gland > intestine > bones > eyeball > gallbladder > eggs > stomach > heart > muscle > brain > lung > blood > uropygial gland > skin > subcutaneous fat. The main studies are performed in genus Larus from Northern Hemisphere. Feathers were the main biological tissue that accumulated this metal. Moreover, we can conclude that standardized non-lethal methodology for measuring metals in biotic matrices can be an adequate methodology to compare data at global scale. In order to understand the effects of metals on gulls, more studies from the Southern Hemisphere are urged. Acknowledgements: Winfred E. Espejo is a scholarship CONICYT-Chile for PhD studies. This study was financially supported by FONDECYT-Chile 1140466 granted to R. Barra and FONDECYT 1140164.

TP274 Impaired swim bladder inflation in early-life stage fathead minnows exposed to a deiodinase inhibitor, iopanoic acid

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The thyroid axis plays a critical role in teleost fish development. The present study investigated whether inhibition of deiodinase, the enzyme which converts thyroxine (T4), to the more biologically-active form, 3,5,3'-triiodothyronine (T3), would impact inflation of the posterior and/or anterior swim bladder (SB), processes which we previously demonstrated to be thyroid-hormone regulated. Two experiments were conducted using iopanoic acid (IOP), a pharmaceutical used to treat hyperthyroidism, as a model deiodinase inhibitor. In the first study, fathead minnow (Pimephales promelas) embryos (~1 day post-fertilization [dpf]) were exposed in a flow-through system to three concentrations of IOP (0.6, 1.9, 6.0 mg/L) or control water and sampled at 4 and 6 dpf. Whole body T4 and T3 concentrations were measured using LC-MS/MS. Abundance of deiodinase 1-3 (dio1-3), thyroid-stimulating hormone (tsh), and thyroperoxidase (tpo) transcripts was examined using quantitative polymerase-chain reaction. Posterior SB inflation was assessed at 6 dpf. To examine effects on anterior SB inflation, a second study was conducted in which 6 dpf larvae, whose posterior SB had already inflated, were exposed to the same IOP concentrations. Fish were sampled at 10, 14, 18, and 21 dpf for T4/T3 measurements, gene transcription analyses, and thyroid histopathology. In the embryo study, incidence and length of inflated posterior SBs were significantly reduced in the 6.0 mg/L treatment at 6 dpf. Dose-dependent increases in T4 and T3 concentrations were measured at 4 dpf, but only T3 concentrations remained elevated until 6 dpf. Significant dose-dependent down-regulation of tpo transcript abundance was observed at 6 dpf. Incidence and length of anterior SB inflation in larval fish were significantly reduced in all IOP treatments at 14 dpf. However by 18 dpf, anterior SBs had inflated in fish from all treatments, suggesting inflation was delayed, not prevented. Significant up-regulation of dio2 mRNA was observed at 14, 18, and 21 dpf, and significant down-regulation of tpo mRNA was observed at 10, 14, and 18 dpf in all IOP treatments. Overall, exposure to IOP altered thyroid hormone concentrations, gene transcription, and swim bladder inflation in early-life stage fathead minnows. The contents of this abstract neither constitute nor necessarily reflect USEPA policy.

TP275 The effects of early-life stage thyroid disruption on morphology, thyroid signaling, and reproduction in fathead minnows (Pimephales promelas)

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The thyroid hormone signaling pathway has been a major focus of research into the effects of endocrine disrupting compounds (EDCs). Thyroid disruptors are typically associated with alterations in growth, development, and metabolism and elicit their effects by binding to thyroid hormone receptors and transport proteins or interfering with hormone synthesis. This study examined the effects thyroid disruption in the fathead minnow (Pimephales promelas), a commonly used model organism for EDC screening and toxicity testing. Specifically, this study sought to assess the impacts of early life stage thyroid disruption on growth, thyroid hormone signaling, reproductive endocrine signaling, and reproductive success in minnows. Newly-hatched fathead minnows were exposed to either thyroxine (T_4) or propylthiouracil (PTU) for 42 days to induce hyper- and hypothyroidism, respectively. At the end of the exposure, the length, weight, and overall physical appearance (i.e., body shape and pigmentation) of exposed fish was recorded and gene expression analysis was performed to identify alterations in thyroid hormone signaling (e.g., thyroid hormone receptors, transthyretin, deiodinases, etc.), and sex steroid hormone signaling (e.g., aromatase, estrogen receptor, etc.). In addition, a subset of fish were transferred to clean water and raised to sexual maturity for the assessment of reproductive performance. It was found that thyroid disruption leads to significant changes in the growth, morphology, and expression of genes related to thyroid hormone signaling. Overall, this study represents an effort to better understand the effects of thyroid disruption in a common model species and provides information that will aid in making predictions about mechanisms of thyroid EDC action on early life stage organism.

TP276 Evaluation of the toxic effect of 2 analgesics in juvenile of zebrafish Danio rerio

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Nonsteroidal anti-inflammatory analgesics, such as aspirin and paracetamol are important pharmaceuticals worldwide, with an estimated annual production of several kilotons. These drugs are sold freely and are substances that are eliminated in considerable amounts aquatic systems it is detected in concentrations 0.001-5 mg L^{-1}. The purpose of this study was to evaluate the toxicity of two analgesics (Paracetamol and Acetylsalicylic acid) in juvenile of zebrafish. Static bioassays were performed with a duration of 96 hours, where five drug concentrations were tested to determine the LC_{50}. Subsequently a chronic test with duration of 22 days was performed exposing the fish to two sublethal concentrations (LC_1 and LC_{10}) to evaluate the following responses: increase in weight rate and 3 biomarkers: lipoperoxidation, Ache activity and genotoxicity (micronucleus frequency). The results showed that the most toxic compound was acetylsalicylic acid (LC_{50} = 34.9 mg L^{-1}). Organisms exposed to sublethal concentrations of analgesics showed a decrease in weight ranged from 23 to 66%. Also a decrease in activity of AChE enzyme between 12 to 34% was observed. The degree of lipid peroxidation ranged from 112.3 to 85.2 and 51.8 to 58.7 nM MDA g^{-1} on tests with Paracetamol and Acetylsalicylic acid respectively (control = 18.2 nM MDA g^{-1}). Micronucleus frequency was high in tests with acetylsalicylic acid, indicating that probably have a genotoxic effect. According to the results we can conclude that both drugs affect the juveniles of Danio rerio, and probably cause adverse to other species of aquatic organisms that are exposed to these medications.

TP277 The effects of Ibuprofen, Naproxen, and 17α-ethinylestradiol on American Flagfish (Jordanella floridae)

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Pharmaceuticals are a very diverse group of bioactive compounds that are frequently detected in the environment. Steroid hormones and non-steroidal anti-inflammatory drugs are often detected due to their high level of use. More knowledge regarding the impacts of environmentally relevant concentrations of pharmaceuticals alone and in mixture are required. American flagfish (Jordanella floridae) were exposed to ibuprofen (0.1 µg/L), naproxen (0.1 µg/L), and 17α-ethinylestradiol (10 ng/L) alone, and in a mixture. Flagfish were placed into breeding harems with 4 females and 2 males per treatment and both a pre-exposure phase (21-d) and an exposure phase (21-d) were completed. Total length, wet weight, liversomatic index, and gonadosomatic index were assessed and had no significant differences. Fertilization, hatchability, and egg production were also monitored. Flagfish exposed to 0.1 µg/L naproxen, and 10 ng/L 17α-ethinylestradiol had a significant decrease ($p \leq 0.05$) in fertilization. 17α-ethinylestradiol (10 ng/L) had a significant decrease ($p \leq 0.05$) in hatchability and average cumulative egg production for ibuprofen (0.1 µg/L) was significantly increased ($p \leq 0.05$). The reproductive effects of 17α-ethinylestradiol are well known, but effects of naproxen and ibuprofen are much less so. These findings suggest that more research is needed to assess the potential reproductive effects of naproxen and ibuprofen on fish and other aquatic organisms.

TP278 An investigation of the mechanisms of action of ethinylestradiol and ammonia on fathead minnow (Pimephales promelas) reproduction

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Understanding the mechanisms of action of individual toxicants and how combined effects manifest at the level of the individual may improve our knowledge of multiple-stressor impacts on fish populations. Complex, municipal effluents in the aquatic environment often are a source of the synthetic estrogen, 17a-ethinylestradiol (EE2) and ammonia (NH3); both compounds are known to negatively affect fish reproduction. This study employed a flow-through exposure of fathead minnows to target concentrations of EE2 (10 ng/L) and NH3 (3 mg/L) alone and in combination to investigate the hormonal and molecular effects of treatment on spawning inhibition. The pre-exposure and exposure phases of the experiment were 25 and 15 days, respectively. LC-MS-MS analyses confirmed the EE2 exposure levels (ng/L) to be 8.2 ± 0.31 and 8.4 ± 0.23 in the EE2 and EE2+NH3 treatments. NH3 was quantifiable in all treatments due to the nitrogenous waste cycle of the fish but was significantly higher in the treated groups with average total NH3 levels (mg/L) of 2.73 ± 0.21 (NH3) and 3.18 ± 0.10 (EE2+NH3). The corresponding levels of calculated unionized ammonia (UIA) in these treatments were 0.36 and 0.42 mg/L. As expected, both treatments containing EE2 and NH3 alone significantly reduced egg production by 84% and 46%, respectively. Liver and gonadosomatic indices were unaffected, but all treatments significantly reduced the condition factor of male and female fish. The average length, but not body weight, of exposed fish was significantly greater in treated fish. Ovarian estradiol levels were reduced 3.5 and 2 fold by EE2 and EE2+NH3 treatments, but NH3 alone had no effect. A suite of genes related to steroid biosynthesis and growth were examined by qPCR. There were increases in ovarian mRNA levels of receptors for growth hormone (GH) and IGF1 by EE2 and NH3 treatments, but GH and ERa receptor mRNA were reduced by all treatments in the female liver. Males exposed to the EE2 containing treatments showed increases in liver vitellogenin mRNA. Molecular analyses are ongoing to further our knowledge of the genes involved in growth and reproduction that may be altered by EE2 or NH3. This study demonstrated that both EE2 and NH3 negatively impact egg production, but their differential effects on ovarian steroid synthesis and their overlapping effects on gene expression highlight the complex nature of delineating the mechanisms behind whole organism responses to environmental contaminants.

TP279 Low-dose exposure to 17alpha-ethinylestradiol rapidly regulates expression networks of gonad differentiation in the adult fathead minnow testes

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In wild fish inhabiting wastewater effluent with estrogenic components, the presence of both oocytes and spermatogenic tissue in the testes has been documented. However, the molecular networks underlying the manifestation of the intersex condition are not completely characterized. Here, we used an environmentally relevant concentration of 17alpha-ethinylestradiol (EE2), the active component of the birth control pill, to determine the response of the fathead minnow testis transcriptome after 96 hrs. The goal was to capture early molecular initiating events that may lead to the intersex condition. There were no effects of EE2 on gonadosomatic index nor proportion of gametes within the testes. However, in vitro production of 11-ketotestosterone and testosterone in the testis was decreased relative to controls following EE2 exposure. Gene expression profiling revealed that there were 10 transcripts that were differentially expressed in the testis using a 8x60K fathead minnow microarray, the most dramatic change being that of coagulation factor XIII A chain (20-fold increase). Guanine nucleotide binding protein (Beta Polypeptide 2), peroxisome proliferator-activated receptor delta, and WNK lysine deficient protein kinase 1a, were down-regulated by EE2. Subnetwork enrichment analysis revealed that EE2 suppressed transcriptional networks associated with steroid metabolism, hormone biosynthesis, and sperm mobility. Most interesting was that gene networks associated with doublesex and mab-3 related transcription factor 1 (dmrt1) were suppressed, despite the fact that dnmt1 expression itself was not different from controls. Moreover cell processes involving forkhead box L2 (foxL2) such as ovarian follicle development and granulosa cell development were increased in the testis. Cell processes that included glucose homeostasis, response to heavy metal, amino acid catabolism, and the cyclooxygenase pathway were decreased with EE2 while lymphocyte chemotaxis, intermediate filament polymerization, glucocorticoid metabolism, carbohydrate utilization, and anterior/posterior axis specification were increased. These data demonstrate that a low dose of EE2 rapidly suppresses male hormone secretion and gene networks related to male sex differentiation, and induces transcriptional networks related to female sexual differentiation in the adult testis. These responses are hypothesized to be the molecular initiating events that occur prior to the development of the intersex phenotype after prolonged exposures to estrogens.

TP280 Exposure to the Contraceptive Progestin, Levonorgestrel, Disrupts Reproductive Behavior, Fecundity, and Morphology in the Fathead Minnow

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Historically, endocrine disrupting chemical research has focused on environmental androgens, estrogens, thyroid hormones, and their antagonists. Recent efforts have begun to examine the effects of gestagens, which include endogenous progestogens and synthetic progestins, on aquatic wildlife. Progestins have been measured in wastewater treatment plant effluent, and exposure studies have demonstrated profound effects on the fecundity, morphology, and most recently reproductive behavior of fish and amphibians. In this study, fathead minnows (Pimephales promelas) were exposed to environmentally relevant concentrations of levonorgestrel (LNG) using a flow-through exposure system consisting of thirty-two 15 L glass tanks (8 tanks per treatment, 6 full volume turnovers/d). One reproductively active triad (two females and one male) was placed into each tank containing a single breeding tile and acclimated for 7 d. Treatments began on day 8, with each tank receiving H_20 only, EtOH (vehicle control), 10 ng/L, or 100 ng/L of LNG. Fecundity (defined as the number of eggs laid) was quantified daily. To determine effects on reproductive behavior, a 1 h recording was obtained on days 1, 2, and 8 from each tank using a front mounted webcam, and various behaviors (lateral displays, lateral quivers, rubbing of the tile ceiling, etc.) quantified using Jwatcher™ (V 1.0). After 8 d of exposure, all fish were euthanized and examined for the presence of secondary sexual characteristics, including nuptial tubercles, dorsal fin spots, and fatpads. Exposure to both concentrations of LNG decreased fecundity, with complete cessation of egg deposition observed on day 5. LNG exposure also caused morphological masculinization of females, indicated by the development of nuptial tubercles, dorsal fin spots, and fatpads. Both LNG treatments caused decreases in approach and leading behaviors on day 1, with significant decreases of all behaviors observed on days 2 and 8. Together, results from this study, androgen receptor activation research in our lab, and research by others indicate that LNG functions at least as an environmental androgen in fishes. As alterations of normal reproductive behavior were observed after just one day of exposure, this study also provides further support that behavior is an extremely sensitive endpoint that merits increased attention in future aquatic toxicology studies.

TP281 Exposure effects of the cyprinid progestogen pheromone, DHP, on the reproductive behavior and sperm characteristics of the male fathead minnow

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In fish, pheromones stimulate reproduction by way of heightening sexual attraction and/or stimulating gametogenesis. In carp and goldfish, several pheromones have been identified including DHP, androstenedione, and prostaglandins. In these species, DHP (17α,20β-dihydroxy-4-pregnen-3one) excreted by ovulating females affects reproductive behavioral and physiological changes in males, and has been hypothesized to function similarly in related species such as the fathead minnow (FHM, Pimephales promelas). Because of the easily identifiable reproductive behaviors of the FHM and its broad use in aquatic toxicology, this species was used to test the effects of DHP on male FHM nest tending behavior and sperm motility. Singly-housed, adult, male FHMs (n=7) were exposed to an ethanol control, 1 or 10 ng/L of DHP overnight using a flow-through system. Exposure began at 6 pm and reached nominal concentrations for each treatment at approximately 12 am, after which dosing was ceased and DHP concentrations allowed to decrease through dilution with water only. Water samples were collected at 6 pm, 10 pm, 12 am, and 8 am and analyzed using LC/MS/MS. At 8 am, 1 h video recordings of each tank were obtained and analyzed for % active time, % static time, and frequency and duration of nest tending behaviors using JWatcher^{TM} (V1.0). Four randomly selected males per treatment were then euthanized, and semen samples obtained via testicular maceration. Total % motility (MOT), average path velocity (VAP), straight line velocity (VSL), curvilinear velocity (VCL), linearity (LIN), and beat cross frequency (BCF) were quantified using computer assisted sperm analysis (Hamilton Thorne). Both concentrations of DHP resulted in increased activity inside the breeding structure, and males exposed to the 10 ng/L treatment also spent more time exploring outside of the tile. Spermatozoa from fish treated with both concentrations of DHP exhibited greater MOT, VAP, and VCL, as well as decreased LIN. No differences between any treatments were observed for BCF and VSL. Our results suggest that DHP causes increased activity in males, and at higher concentrations may initiate searching behavior for ovulating females. As several endocrine disrupting chemicals have been shown to bind the same receptors as DHP and greatly affect fecundity, further research is required to determine if these compounds can also function via the disruption of pheromonal communication.

TP282 Developmental toxicity of sulfamethazine in marine medaka (Oryzias melastigma) embryos

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Sulfamethazine (SMZ) is not only extensive used in treatment of human diseases, but also largely used as the long-term additive in animal feed. It has been a kind of ubiquitous contaminants in the marine environment. Bioaccumulaition and potential developmental toxicity of SMZ in marie madaka (Oryzias melastigma) embryow were studied. Results indicated that bioaccumulation of SMZ in embryos showed a significant dose-response relationship. The main malformation in embryoic stage was hemoglutination, and the region and manifestation of hemoglutination were irregular. Yolk sac edema was the main malformation at the larvae stage. The trend of embryonic mortality was so similar with the trend of hemoglutination rate, implied the hemoglutination was proably the main cause of embryonic death. The embyonic heart beats increased with SMZ concentrations on 4 dpf and 12 dpf. Suffering from exposure to SMZ, superoxide dismutase (SOD) and catalase (CAT) activities of embryos showed a significant dose-effect relationship. When the concnetrations of SMZ in embryos reached to 10 ng/embryo, SOD activiiesy were significant inhibited and caused oxidtive lessions. These results indicated that fish at the early develomental stage were sensitive to antibiotic and the rational administration of drugs in fries aquaculture should be concerned.

TP283 The Effects of Risperidone, an Antipsychotic, on American Flagfish (Jordanella floridae) Reproduction

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Concern of pharmaceuticals and personal care products (PPCPs) in the environment has increased in the last decade. Most research concerning fish reproduction been focused on pharmaceuticals that directly affect the endocrine system. The goal of this study was to understand the effects of a non-endocrine active pharmaceutical, risperidone, on fish reproduction using American flagfish (Jordanella floridae). Risperidone is an antipsychotic drug whose side effects in mammals include increased prolactin levels from inhibiting dopamine signaling. Prolactin's role in reproduction is understudied in fish; however, prolactin may impact reproduction through modulation of steroid hormones and gonad development and maturation. Additionally, risperidone inhibition of dopamine signaling has caused low libido and sexual dysfunction in mammals. Risperidone has been detected in surface waters at concentrations in the low ng/L range. Sexually mature American flagfish were exposed to 1, 10, and 100 ng/L risperidone for 21 days in breeding harems (2 males:4 females). Risperidone exposure did not adversely affect with fecundity, fertilization, or hatching success. The results of the present study suggest that risperidone exposure at environmental levels may not pose a significant risk to fish reproduction.

TP284 Effect of carbamazepine and valproate exposure on development of zebrafish (Danio rerio) embryos

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Carbamazepine (CBZ) and valproic acid (VP) are wide utilized in epilepsy treatment with good results in seizure control, but the exposure to this compounds result in a risk factor on developing, therefore, in many countries low levels of these compounds have been detected in treatment plant effluents, surface waters, seawaters, groundwater and some drinking waters. A fast and simple method to study chemical toxicity after exposure of the complete vertebrate embryo during embryogenesis in ovo is the zebrafish embryotoxicity test (ZET). In this study, we determinate the teratogenic index (TI) and embryotoxic effect with respect to a general morphology scoring (GMS) of two anticonvulsant drugs, CBZ and VP, using an easy, fast and semiquantitative method. Embryos were exposed to four concentrations of CBZ (62.5, 125, 250 and 500 μM) and VP (12.5, 25, 50 and 100 μM) in a static exposure systems for 12, 24, 48 and 72 h. Results showed that in CBZ treatment only in highest concentration (500 μM) presents pericardial edema, with a TI of 0.016. VP groups present pericardial edema, heart deformation, scoliosis and deformation yolk in high concentrations (50 and 100 μM), the minimal TI was found in 25 μM (0.016), increasing in 50 μM (0.13) and 100 μM (0.23). V group has a mortality of 10.42%, while in CBZ, the maximum mortality were 14.58% in 62.5 μM and VP showed a concentration-dependent behavior, with 25 % in 50 μM and 96.87 % in 100 μM of death embryos. CBZ showed hatching delay and pericardial edema in high concentration (500 μM), with a low incidence of defects. VP showed high toxicity producing abnormalities as pericardial edema, scoliosis, tail deformation, absence of pectoral fins and wide mortality. We concluded that CBZ and VP are two pharmaceutical products that could affect the normal aquatic fauna development.

TP285 The combinatorial effects of Hydroxypropyl-β-cyclodextrin (HPβCD) and synthetic steroids on American Flagfish growth and reproduction

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The risk of pharmaceuticals and personal care products (PPCPs) in the aquatic environment has been a concern for the past two decades. Many studies have demonstrated the growth and reproductive toxicity of PPCPs to non-target aquatic organisms. The aims of this study were to firstly, understand the toxicity of commonly consumed synthetic steroids, 17α-ethinylestradiol (EE2), levonorgestrel (LNG), and etonogestrel (ENG) on the American flagfish (Jordanella floridae). Secondly, to determine if the odour suppressant/excipient, hydroxypropyl-β-cyclodextrin (HPβCD) can alter the identified toxicity of EE2, LNG, or ENG. EE2 is a synthetic estrogen, while LNG and ENG are both synthetic progestins, all of which are commonly found in oral contraceptives. In current literature, both EE2 and LNG have demonstrated the ability to alter early life-stage growth and adult reproduction. To date, ENG has not been studied in an environmental toxicological setting, however, it is of high interest due to its similar physio chemical properties to LNG, including a high relative binding affinity for the human progesterone receptor. HPβCD is both used as an excipient in the pharmaceutical industry as well as an odour suppressant in Febreze®. HPβCD is amphiphilic and toroidal in shape with the ability to include non-polar compounds within its central cavity, including many synthetic steroids. Results from this study indicate that in the presence of HPβCD, the 96 hr acute toxicity of EE2 to larval American flagfish was significantly reduced in a 1:1 molar ratio (EE2:HPβCD) ($P \leq 0.05$). Thus an interaction between HPβCD and EE2 can be detected though a biological assay. In order to characterize the environmentally relevant concentrations of these compounds in a chronic setting, a 30 day early life stage toxicity test was undertaken using juvenile American flagfish. Fish were exposed to 1 ng/L, 3.2 ng/L, 10 ng/L, 32 ng/L, 100 ng/L, and 320 ng/L LNG in triplicate. No significant differences in wet (n=96-104) or dry weight (n=30) were observed. A minor increase in length was observed at 32 ng/L LNG when compared to the control ($P \leq 0.05$). Given that the aim of this study is to determine if HPβCD can alter the toxicity of synthetic steroids to fish, eliciting a toxicological response is vital to this study. As such, a series of reproductive exposures involving EE2, LNG, and ENG, alone and in combination with HPβCD are currently being conducted with results to be discussed.

TP286 Transgenerational effects of triclosan on the demography of Brachionus havanaensis (ROTIFERA)

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Triclosan is an antimicrobial agent commonly used worldwide in several personal care products, such as hand soaps. It is an endocrine disruptor that affects a variety of aquatic and terrestrial organisms. It can potentially mimic estrogens, androgens or even thyroid hormones. In Mexico different concentrations of triclosan have been reported in aquatic systems. However, there is no law regulating the presence of chemicals such as triclosan, in aquatic systems. The lack of information about possible effects on aquatic organisms of this chemical has increased concern among ecotoxicologists. Rotifers which are the common components of aquatic ecosystems are sensitive indicators and are frequently used in bioassays. Brachionus havanaensis is a common rotifer in freshwater ecosystems of Mexico and has been used in ecotoxicological bioassays. In this work, the median lethal concentration (LC_{50}, 24h) of Brachionus havanaensis exposed to triclosan was determined. Based on the LC_{50}, we tested three sublethal concentrations of triclosan to quantify the demographic responses of B. havanaensis for two successive generations (F_0, and F_1). The 24 h LC_{50} of triclosan for B. havanaensis was 500 µg L^{-1}. B. havanaensis exposed to sublethal concentrations of triclosan showed that both the survival and reproduction-related variables were negatively affected with increasing concentrations of the endocrine disruptor. There were also significant differences in relation to the demographic responses of B. havanaensis at F_0 and F_1 exposed to triclosan. The impact of this compound on aquatic ecosystems is discussed.

TP287 Early exposure to triclosan impairs heart formation, metamorphosis and reproduction in zebrafish

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Triclosan (TCS) is an antimicrobial agent that is prevalent within surface waters and readily bioaccumulates within aquatic organisms. Here we demonstrate that exposure to environmentally relevant concentrations of TCS during critical periods of development can impair key processes of development in zebrafish. Fish were exposed to concentrations ranging from 0 – 400 ?g TCS/L via static waterborne exposure with daily renewal either from 0-5 days post fertilization (dpf) or from 21 – 35 dpf. While cardiac edema and reduced heart function of embryos were only seen in larvae exposed to high concentrations of TCS (≥ 40 ?g TCS/L), impacts on heart looping and regurgitation were seen following exposure to as little as 0.4 ?g TCS/L. Our preliminary findings suggest that exposure to as little as 0.4 ?g TCS/L may impair ability to capture prey, and may also impair the development of craniofacial structures. Larvae exposed to as little as 0.4 ?g TCS/L showed delayed juvenile development and reductions in egg production. Taken together, this work suggests that chronic exposure to environmentally relevant concentrations of TCS has the potential to impact wild fish populations, and warrants further study.

TP288 Early-life Stage Effects of Metformin in Japanese medaka (Oryzias latipes)

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In recent years, there is a growing concern that human pharmaceutical waste threatens to disrupt many natural processes of living organisms by the introduction of stressors and unnatural contaminants in the environment. One of the most abundant environmental contaminants is the type 2 diabetic drug metformin. Metformin has been measured in surface waters and wastewater effluents in the µg/L concentration range. Recent studies have observed adverse effects of metformin on adult fathead minnows, resulting in an increase in vitellogenin expression, an increase occurrence of intersexuality, a decrease in weight and condition factor in males, and a decrease in the size of amount of egg clutches produced by breeding minnows. To date, there has been no studies investigating the effect of metformin on the sensitive early life stages of aquatic organisms. The present study utilized the Organization for Economic Cooperation and Development (OECD) 210 Early-life Stage (ELS) study to investigate the impacts metformin has on Japanese medaka growth and development. Medaka were treated with a concentration range of 1.0, 3.2, 10, 32 and 100 µg/L metformin from embryo through 21 days post hatch. Results will be presented showing length, weight (wet and dry), and condition factor of fish treated with metformin compared to control fish. Growth and developmental effects of this major pharmaceutical on this important early-life stage will be discussed.

TP289 Dose-dependent impacts of wastewater contaminant metformin on the reproductive system of zebrafish (Danio rerio)

J. Crago, Texas Tech Univ/TIEHH / Environmental Toxicology The Inst of Environmental and Human Health TIEHH; R. Klaper, Univ of Wisconsin-Milwaukee / School of Freshwater Sciences

The discovery of intersex in freshwater fish in the early 1990s suggested the presence of endocrine disrupting compounds in the aquatic ecosystem, likely from human waste. Although synthetic estrogens from birth-control medications found in wastewater have been pointed to as one likely cause of observed endocrine disruption in the environment, other pharmaceuticals and personal care products have been implicated as causing endocrine disruption in fish. One such drug, metformin is prescribed worldwide as a treatment for Type 2 diabetes. This biguanidine is excreted in human waste in its active form and, although it is removed to a high degree by wastewater treatment, recent studies have found this compound in many freshwater systems around the world and it is now estimated to be the pharmaceutical most deposited into the aquatic environment by mass. In our previous work we found evidence of intersex in males and gene expression changes in fathead minnows (Pimephales promelas) exposed to 40 ppb of metformin from fry stage to adulthood. For this talk, we have continued to examine metformin as well as its main wastewater treatment metabolite guanylurea. We exposed zebrafish (Danio rerio) to 0.4, 4 or 40 ppb of metformin from 6 hours post fertilization to 5 months and then performed a 21-day reproductive study. Zebrafish exposed to 4 and 40 ppb metformin had a reduction in egg production as compared to control and 0.4 ppb exposed metformin. There was no difference in egg production in zebrafish exposed to guanylurea versus the control. As part of this study, we also found genes associated with reproduction and steroidogenesis were altered in response to exposure to metformin and guanylurea. This study will describe the potential impacts of metformin and guanylurea as a non-traditional endocrine disrupting compound.

TP290 Evaluation of the effects of chronic exposure to fluoxetine (Prozac) and norfluoxetine on the benthic macroinvertebrate Chironomus riparius

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Approximately half of those prescribed an antidepressant, for conditions such as depression, anxiety and eating disorders, are given a Selective Serotonin Reuptake Inhibitor (SSRI), such as Prozac (fluoxetine, FLX). Interestingly, a large percentage of FLX, and its metabolite, norfluoxetine (NFLX), are excreted from the body. Due to incomplete removal of FLX and NFLX during wastewater treatment, FLX has been identified in surface waters receiving municipal effluent within the U.S., with levels reaching up to 1.4 ppb. The FLX and NFLX present in waterways are likely to adsorb to aquatic sediment, due to their lipophilic properties. The benthic macroinvertebrate, Chironomidae, has been used in several studies to test the effects of FLX on reproduction, development and gene expression, with conflicting results. There has been limited research on NFLX's effect on aquatic organisms, even though it is thought to be more biologically active and has a longer half-life than FLX. Our research examines changes in growth, emergence timing, survival, reproduction, fecundity and generational survival in Chironomus riparius due to exposure to varying levels of fluoxetine or norfluoxetine. We have found that FLX and NFLX negatively affect survival, in concentration ranges of 5-18 ppm and 1-6 ppm respectively. NFLX, but not FLX, reduces emergence timing and reproduction. We are currently evaluating if chronic exposure is significantly affecting growth or generational survival, as well as determining if the FLX and NFLX are accumulating in the larvae. In addition to developmental effects on Chironomidae, we are determining larval behavioral consequences due to chronic exposure to FLX and NFLX, by evaluating the rate of movement and distance traveled in both light and dark conditions. Analysis of the effect of SSRI contamination on ecological integrity will influence water quality regulations.

TP291 Effect of selective serotonin reuptake inhibitor (SSRI) citalopram on hybrid striped bass predatory behavior and brain chemistry

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Antidepressants are commonly prescribed drugs in the United States. Selective serotonin reuptake inhibitors (SSRIs) are one of the most prescribed antidepressant classes. Waste water treatment facilities are often ill-equipped to handle the removal of pharmaceuticals from incoming waters, leading to the discharge of SSRIs into the environment. Fish populations around discharge pipes could be experiencing exposures to sub-lethal concentrations (low ppb to high ppm) of SSRIs which could affect their ecological fitness in the environment. The goal of this research is to determine whether antidepressants have a sublethal effect on fish populations through a change in feeding behavior, further quantified by analyzing brain chemistry. Past research of SSRIs on hybrid striped bass (HSB) showed a decrease in serotonin concentrations in the brain and concentrations of SSRIs equivalent to therapeutic levels in humans. We hypothesize a decrease in feeding behavior and decrease in serotonin levels in HSB after exposure to citalopram. HSB were exposed to citalopram concentrations ranging from 50-150 ug/L followed by feeding periods every three days (3,6,9,and 12 days) to determine if citalopram has an effect on their predatory behavior. Blood and brain samples collected from euthanized fish at the end of an exposure were analyzed for concentrations of citalopram and serotonin. Preliminary work has shown medium and high exposure to citalopram may cause a decrease in feeding ability and/or causing satiation. From an ecological standpoint an increased feeding time could make exposed bass populations less ecologically fit compared to other fish populations not as affected by the antidepressants.

TP292 The effects of atorvastatin at environmentally relevant concentrations on steroidogenesis of the Western African Clawed frog (Xenopus tropicalis)

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Micropollutant pharmaceuticals (MP) have become an emerging threat to aquatic ecosystems. Atorvastatin has been detected in wastewater effluent at a variety of levels from 1 ng L^{-1} to 0.47 µg L^{-1}. Statin drugs work to inhibit endogenous production of cholesterol by inhibiting 3-hydroxy-3-methyl-glutaryl-CoA reductase from producing mevalonic acid in the mevalonate pathway. The reduction in circulating cholesterol levels may affect steriodogenesis by reducing the levels of estrogen and testosterone produced in the steroidogenesis pathway. Due to the increased sensitivity of amphibians to aquatic toxins, we hypothesize that the frog population could be significantly impacted by interruption of endogenous synthesis of cholesterol which may lead to altered steroidogenesis due to low concentrations of atorvastatin in wastewater effluent. The central hypothesis of this study is to directly address this issues by using a combination of chronic exposure bioassays at critical life stages to assess the potential for atorvastatin to cause overt reproductive and developmental dysfunction to wetland amphibians. Xenopus tropicalis tadpoles will be exposed to 5 environmentally relevant concentrations at critical development stages. A chronic 21 day exposure will evaluate the effects during early life exposures from fertilization to free swimming tadpole stages. A chronic 30 day exposure will evaluate effects during metamorphosis and growing into adulthood. These two exposures combined allow for evaluation of the complete lifecycle from fertilization to adulthood. The adult reproductive exposure will be performed by injecting adults with relevant concentrations of atorvastatin, then breeding the injected pairs. The offspring will then be evaluated for effects of atorvastatin exposure resulting from parental exposure. This research will elucidate the effects of MPs in wastewater effluent on the growth and development of a model amphibian.

TP293 Marine Bivalve Cellular Responses to Beta Blocker Exposures

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β blockers are prescription drugs used for medical treatment of hypertension and arrhythmias. They prevent binding of agonists such as catecholamines to β adrenoceptors. In the absence of agonist induced activation of the receptor, adenylate cyclase is not activated which in turn limits cAMP production and protein kinase A activation, preventing increases in blood pressure and arrhythmias. After being taken therapeutically, commonly prescribed β blockers may make their way to coastal habitats via discharge from waste water treatment plants (WWTP) posing a potential risk to aquatic organisms. The aim of our research is to evaluate cellular responses of three commercially important marine bivalves – Eastern oysters, blue mussels and hard clams – upon exposure to two β blocker drugs, propranolol and metoprolol, and to find molecular initiating events (MIEs) indicative of the exposure. Bivalves were obtained from Narragansett Bay (Rhode Island, USA) and acclimated in the laboratory. Following acclimation, gills and hepatopancreas (HP) tissues were harvested and separately exposed to 0, 1, 10, 100 and 1000 ng/l of each drug. Tissues were bathed in 30 parts per thousand (ppt) filtered seawater, antibiotic mix, Leibovitz nutrient media, and the test drug. Exposures were conducted for 24 hours and samples were saved for cellular biomarker assays. A lysosomal destabilization assay, which is a marker of membrane damage, was also performed at the end of each exposure. EC50 values were calculated and compared across species to evaluate drug and dosage effects as well as species sensitivities. Exposed tissues had significantly higher membrane damage than controls at environmentally relevant concentrations. Differences in species sensitivities were also observed. The results suggest that propranolol is more toxic than metoprolol in all three species. Cellular damage and enzymatic markers are currently being analyzed. These studies enhance our understanding of the impacts of commonly used prescription medication on organisms in coastal ecosystems and demonstrate that filter feeders such as these marine bivalves can serve as good model organisms to examine the effects of water soluble drugs like propranolol and metoprolol. Evaluating a suite of biomarkers allows us to better define MIEs that can be used to develop adverse outcome pathways (AOPs) for environmental exposure to β blockers.

TP294 Effects of exposure to two organic ultraviolet filters and a related commercial sunscreen product in adult coral nubbins

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Ethylhexylmethoxy-cinnamate (EHMC) and octocrylene (OC) are two widely used organic ultraviolet (UV) filters added to personal care products (e.g. sunscreens) to protect skin from sunburn and cancers caused by UV radiation. They possess relatively high octanol-water partition coefficients (Log Kow) and bio-concentration factors (BCF), and are two of the most prevalent organic UV filters found in environmental samples. Previous studies reported that organic UV filters and sunscreen products caused coral bleaching at low concentrations/quantities. Coral reefs are known to be one of the most important ecosystems on Earth. Despite the potential negative effects of organic UV filters and sunscreens on the health of corals, information is lacking about their toxicities. In this study, seven-day exposures were conducted to evaluate the toxicities of EHMC, OC and a related sunscreen product containing both compounds [EHMC, 7% w/w; OC, 3.6% w/w] to two coral species, Seriatopora caliendrum and Pocillopora damicornis. The tested concentrations of EHMC and OC ranged from 0.1 to 1000 µg/L. The sunscreen product was tested by applying it to the hands of volunteers (2 mg/cm^2), after which the volunteers waved their hands gently in 2L filtered seawater for 20 minutes. Serial dilutions of 5%, 20%, and 50% were prepared and were tested along with 0% and 100% treatments. Three replicates of 3 nubbins were prepared in glass bottles, and mortality, bleaching, and polyp retraction were measured. After seven days, death (33.3%) and bleaching (83.3%) of S. caliendrum nubbins were found in the 1000 µg/L EHMC treatment. Thorough polyp retraction was the only adverse effect found in P. damicornis exposed to EHMC; this effect was found in both S. caliendrum and P. damicornis exposed to OC. In the sunscreen product exposures, 5% sunscreen water caused death of S. caliendrum (66.7%) and P. damicornis (33.3%); chemical analysis showed that the 5% sunscreen water contained approximately 400 µg/L EHMC and 30 µg/L OC at the beginning. Based on all of these results, EHMC and OC may not be the major contributors causing the effects of this sunscreen product on S. caliendrum and P. damicornis nubbins. The results of a probabilistic risk assessment showed that EHMC poses high risks to the health of corals in Hong Kong and Taiwan. In the future, more studies should be conducted to investigate the toxicities of organic UV filters and sunscreen products to corals.

TP295 Genotoxicity assessment of mixtures of veterinary antibiotics to fish through the micronucleus test and other nuclear abnormalities

R. Botelho, Univ of São Paulo / Ecotoxicology; J. Correia, UNESP Univ Estadual Paulista Júlio de Mesquita Filho / Biology; C.A. Christofoletti, Fundacao Herminio Ometto / Environmental Mutagenesis; Y. Ansoar, São Paulo State Univ UNESP / Biology; V.L. Tornisielo, CENA Center for Nuclear Energy in Agriculture

Veterinary antibiotics are one of the most used pharmaceuticals class in Brazilian fish farming for the treatment of bacterial diseases, and because of their intensive use, contamination of the aquatic environment may occur with effects to the native biota which lives where the fish farming is located. The goal of this study was to investigate the genotoxic effects of mixtures of environmental concentration of oxytetracycline and florfenicol and the mixtures of their half and double on juveniles of native fish from brazil (Prochilodus lineatus) through the occurrence of micronucleus (MN) and other nuclear abnormalities (NA) after 96 hours exposure. Environmental concentration of oxytetracycline (8000 ng L-1) and florfenicol (425 ng L-1) were determined in surface water of a fish farm located at Ilha Solteira Reservoir (Santa Fé do Sul, São Paulo, Brazil) through Online-Solid-Phase Extraction coupled to Liquid Chromatography-Tandem Mass Spectrometry (Online-SPE-LC/MS-MS) in a previous study of our laboratory and exposed (8000 ng L-1 + 425 ng L-1) along with its double (16000 ng L-1 + 850 ng L-1) and its half (4000 ng L-1 + 212.5 ng L-1) on juveniles of P. lineatus during 96 hours in a static system. Tee genotoxic results are being analyzed and will be presented at the event.

TP296 Genotoxicity assessment of mixtures of veterinary antibiotics to fish through the comet assay

R. Botelho, Univ of São Paulo / Ecotoxicology; C.A. Christofoletti, Fundacao Herminio Ometto / Environmental Mutagenesis; J. Correia, UNESP Univ Estadual Paulista Júlio de Mesquita Filho / Biology; Y. Ansoar, São Paulo State Univ UNESP / Biology; V.L. Tornisielo, CENA Center for Nuclear Energy in Agriculture

Florfenicol and oxytetracycline are the two most commonly used antibiotics for bacterial treatment in fish farming in Brazil, and because of their intensive use, the potential harmful effects on aquatic organisms are of great concern. The goal of this study was to investigate the genotoxic effects of mixtures of environmental concentration of oxytetracycline and florfenicol and the mixtures of their half and double on juveniles of native fish from brazil (Prochilodus lineatus) through the comet assay after 96 hours exposure. Environmental concentration of oxytetracycline (8000 ng L-1) and florfenicol (425 ng L-1) were determined in surface water of a fish farm located at Ilha Solteira Reservoir (Santa Fé do Sul, São Paulo, Brazil) through Online-Solid-Phase Extraction coupled to Liquid Chromatography-Tandem Mass Spectrometry (Online-SPE-LC/MS-MS) in a previous study of our laboratory and exposed (8000 ng L-1 + 425 ng L-1) along with its double (16000 ng L-1 + 850 ng L-1) and its half (4000 ng L-1 + 212.5 ng L-1) on juveniles of P. lineatus during 96 hours in a static system. The genotoxic results are being analyzed and will be presented at the event.

Environmental or Analytical Chemistry – Poster Only – Part 2

TP297 A computational study of nucleophilic aromatic substitution reaction of 3-5-dinitroethoxylpyridine with piperidine using hybrid dft method

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This report involves a reaction between 3,5-dinitroethoxylpyridine and piperidine to give 3,5-dinitro(2-piperidinyl)pyridine. This reaction was carried out using density functional method at the B3PW91, 6-31G level of theory. The mechanism of this reaction begins with the attack on the leaving group (ethoxyl ion) in the compound 3,5-dinitroethoxylpyridine by the nucleophile; piperidine to form an unstable intermediate called Meisenheimer complex. When this intermediate is formed, the reaction can take either of two pathways; based-catalyzed pathway and base-uncatalysed pathway. The base-uncatalysed pathway is a direct decomposition of the intermediate to the product. The leaving group; ethoxyl ion and a hydrogen atom from the intermediate cleave off to form ethanol and leaves as a byproduct, the aromaticity of the compound is then restored. While for the base-catalysed pathway, a strong based is reacted with the intermediate to deprotonate the compound, then the leaving group in the compound cleaves off with a hydrogen atom to form the final product 3,5-dinitro(2-piperidinyl)pyridine. This reaction passes through two transition states going from the reactant to the final product. The first transition states lies between the reactant and the intermediate while second transition state is in-between the intermediate and the product. The structures of these transition states are viewed after optimizing the structures involved in this reaction. The structures were optimized using Gaussian software. The thermodynamics and chemical kinetics of the reaction were gotten using a computer-based model software; Chemcraft. Computational studies can be carried out to find a starting point for a laboratory synthesis, or to assist in understanding experimental data, such as the position and source of spectroscopic peaks. It can also be used to predict the possibility of so far entirely unknown molecules or to explore reaction mechanisms that are not readily studied by experimental means. This study is used in identifying correlations between chemical structures and properties.

TP298 A sensitive and reliable analytical method for free thyroid hormones in animal serum using ultrafiltration in combination with UHPLC-MS/MS

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Thyroid hormones (THs), such as L-thyroxine (T4) and 3,3',5-triiodo-L-thyronine (T3), play critical roles in regulating metabolism and maintaining energy balance in both human beings and animals. There has been a growing interest in animal exposure to environmental pollutants and corresponding adverse effects on thyroid hormone homeostasis. For THs measurement, liquid chromatography-tandem mass spectrometry (LC-MS/MS) has recently attracted attention as an advanced method superior to the conventional immunoassay methods that have been traditionally used. Although almost all THs in blood circulation are bound to transport proteins, approximately 0.03% of the total blood T4 and 0.3% of the total blood T3 are present as protein-unbound (free) form and are able to enter cells followed by physiological effects. For measuring free THs in serum/plasma, physical separation of the free fraction from the mixture of protein-bound and unbound fraction is required prior to quantitative analysis. The aim of the present study was to develop a sensitive and reliable analytical method for the determination of free THs: T4, T3, 3,3',5'-triiodo-L-thyronine (rT3), 3,5-diiodo-L-thyronine (3,5-T2), 3,3'-diiodo-L-thyronine (3,3'-T2), and 3-iodo-L-thyronine (3-T1), in serum/plasma of various animals. As a separation step prior to LC-MS/MS analysis, ultrafiltration (UF) was examined, and UF devises and several experimental conditions (centrifugal force, time, temperature, and serum pH) were optimized and validated. As a result, UF at 1100 g and 37oC for 30 min with a 30 kDa ultrafiltration device (Centrifree YM-30, Millipore) yielded the finest precision (CV: < 10%). There were no statistical differences in measurement values at serum pH values ranging from 7.3 to 8.7. Acceptable accuracy (recovery: 100%–126%) and intra- and inter-day precision (CV: < 10% and < 12%, respectively) were obtained, and method detection limits were between 1.7 and 6.0 pg mL^{-1} serum. The developed analytical method was successfully applied to the determination of free THs in serum/plasma samples of humans, bovines, and cats.

TP299 Analysis of Drugs and Poisons in Catfish

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To ensure the safety of the food supply in the US many organizations are cooperating to develop and improve methods. In cooperation with the USDA/FSIS/FERN the Mississippi State Chemical Laboratory (MSCL) is working to improve the Drug and Poisons Screen using Acidic and Basic Extraction (FERN CHE.0006.01) with GC-MS in catfish and to implement the improved method. The MSCL currently analyzes multiple selected compounds (current used pesticides and metabolites) in farm-raised catfish. A surveillance program within the state supports the growth of the catfish industry in Mississippi and is based upon generating a product that tastes good and is safe for all consumers. The Drug and Poisons Screen has added an additional tool to the laboratory to test catfish for additional compounds (some which have been banned in the US) that may adulterate the edible catfish fillets. The compounds include: arecoline, nicotine, phorate, tetramine, diazinon, terbuphos, paraoxon, parathion, aldrin, pilocarpine, DDE, DDT, endrin, pentazocine, scomolamine, codein, phenylbutazone, morphine, fentanyl, strychnine, phenanthrene, and triphenylphosphate. The Agilent 7890A with the 7000 Triple Quad GC/MS was used for the analysis of the compounds. The FERN method has performed admirably with many different products in the past. The method includes extraction of fish fillets in glycine buffer (pH 3 and pH 10), acetonitrile, NaCl, and centrifugation. The extraction of the catfish fillets causes additional matrix issues that increase problems with the inlet more than other matrices resulting in failures with calibration verifications. The addition of an alumina clean-up column has decreased the matrix effects while maintaining excellent recovery rates for most compounds. A 10 g sample was combined with 10 ml buffer (pH 3 and pH10) and cleaned with 500 mg alumina columns. Recovery of most compounds was > 60%, except pentazocine – 45%, strychnine – 45%, morphine < 10%, and phenylbutaxone < 5% with the pH 10 buffer. Recovery of most compounds was > 60%, except arecoline – 20%, nicotine – < 5%, pilocarpine – 45%, scopolamine – 35%, codeine – 30%, morphine – < 5%, strychnine – 40% with the pH 3 buffer. The pH 3 buffer had more matrix issues than the pH 10 buffer. Additionally the preconditioning of the column with multiple injections of control extract increased the reliability of the results.

TP300 Analysis of Fragrance Ingredients in Fish with Ultrasonic Assistant Extraction and Purge & Trap

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Fragrance materials are used in a wide variety of consumer products including both personal care and household products, such as essential oils. Assessing substances with unknown or variable composition like the essential oils is problematic under REACH, especially assessment of bioaccumulation. In vivo studies are still the 'gold standard' for these assessment, but are hindered by the lack of a simple and reliable analytical method for determining these substances in biota. Herein we present an analytical method for simultaneously measuring a group of model chemicals similar to components of essential oil mixtures with a wide range of volatility property (violate to semi-volatile organic compounds, VOCs – SVOCs) (α-pinene (PIN), camphene (CAM), limonene (LIM), 2-t-butyl-cyclohexyl acetate (Verdox), cashmeran (DPMI), cyclohexyl salicylate (CHS), acetyl cedrene (AC), musk xylene (MX) and globanone (GLO)) in rainbow trout using ultrasonic assistant extraction (UAE) and then a purge and trap (P&T) approach (home-made system), followed by analysis with gas chromatograph – mass spectrometer (GC-MS). Whole rainbow trout were homogenised and subsamples (~ 5 g) were spiked with the test chemicals and surrogate standards. Different organic solvents were tested to extract the compounds with UAE, with acetonitrile as the best choice because of the limited amount of fat extracted and easier subsequent clean up procedures. Gas phase extraction (P&T) was found to be the better method when compared to C18 clean-up method for recovering target chemicals. Hexane was used to exchange the acetonitrile to facilitate the evaporation for the P&T method. Then the P&T procedure was optimised in terms of temperature (50, 70 and 90 °C) and extraction time (0.5 – 310 h), and the best combination was 70 °C for 24 h. The finalized method has been evaluated for the blanks, overall spiked recoveries and robustness. The overall recoveries were 67% (PIN) to 140 % (verdox). While the RSDs of repeated spiked recoveries were in the range of 2.3 % (AC) – 12% (GLO) except CHS (21%) and were within 15% except CHS with 26% for the recoveries in three different weeks. This method is suitable for application to improve bioaccumulation assessment of essential oils.

TP301 Application of graphene-derivatized silica as an efficient solid phase extraction sorbent for enrichment of five polycyclic musks in water

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Pharmaceuticals and personal care products (PPCPs) have gained increasing interest due to their potential risk in aqueous environment. Polycyclic musks (PCMs) which were often detected in water at low concentrations, are considered as important ingredients of PPCPs. To prevent adverse effects on the environment and human health, it is importance to develop a sensitive and fast method for the determination of PCMs in water. This work presents a novel analytical method based on graphene for the determination of PCMs in aqueous matrices. A solid-phase extraction (SPE) adsorbent was synthesized by covalently binding graphene sheet to silica (G@silica) for PCM enrichment in water. This new type of adsorbent was characterized by Fourier transform infrared (FT-IR) spectroscopy, scanning electron microscopy (SEM). Five PCMs (ADBI, AHMI, HHCB AHTN, ATII) were enriched from water samples using 50 mg G@ silica packed cartridge and determined by GC-MS. Various parameters, including eluting solvent and volume, absorbent amount were optimized. Validation experiments showed that the optimized method presented good linearity (r>0.998) in the concentrations ranged from 1 ng/mL to 500 ng/mL. Satisfactory precision (RSD < 10%), and high recovery (79.3-108%) were obtained. The limits of detection were from 0.02 ng/mL to 0.1 ng/mL. This method exhibited the advantages of simplicity, good sensitivity, and high efficiency.

TP302 Assessment subproducts generated from selected disperse dyes bearing azo groups biotransformed by reductase enzyme

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It is known from literature that many environmental pollutants such as azo dyes are mutagenic and suspected of causing cancer. In recent years the result of the intake of these dyes present in drinking water is discussed by several authors. Toxicological risks of synthetic dyes human health are closely related to biotransformation processes enhanced by the mode and time of exposure to human, oral ingestion, skin sensitization, awareness airway and others. One way of azo dyes biotransformation is bacterial biodegradation, which is often initiated with cleavage of azo bonds by azoreductases, which is the key enzyme for the degradation of all azo dyes by bacteria. Therefore, analysis LC-MS/MS were performed to monitor the textile dyes, such as disperse red 73 (DR73), disperse red 78 (DR78) and disperse red 167 (DR167) biotransformation and major metabolites generated. The bacterium E. coli was isolated from different phases of activated sludge produced by the textile company Vicunha, Brazil. Selected strains were inoculated with a low concentration of glucose and mixed with the azo dye in a concentration of 100 mg L^{-1} each dye; and incubated for seven days. LC-MS/MS analysis showed the presence of two metabolites for each dye after the incubation. 3-4-Aminophenyl-ethyl-amino-propanitrile (m/z 190) was detected for both DR73 and DR78. 4-Nitroaniline (m/z 139) and 2-chloro-4-nitroaniline (m/z 173) were also detected for DR73 and DR78, respectively. These results suggested that DR73 lost the CN^- substituent during the biotransformation. The main products from DR167 biotransformation were dimethyl 3,3'-3-acetamido-4-aminophenyl-azanedyl-dipropanoate (m/z 337) and 2-chloro-4-nitroaniline (m/z 173). The detected products were corresponding to amines after the cleavage of the azo bond. All the compounds identified as metabolites of textile dyes have been classified as possibly human carcinogens (2B) by the IARC. Therefore, our findings corroborate with the recommendation of The European Union that prohibits importing and marketing products that could produce carcinogenic amines at levels of ppm (Decision 2002/657/EC). In conclusion, both the original textile dyes and their degradation products may harm the human health and the environment. Considering that the reduction steps used in this work mimic endogenous reactions, toxic compounds could originate in the human liver and gastrointestinal tract after oral exposure to DR 73, DR 78, and DR 167.

TP303 Derivatization, DLLME, and GC-FID analysis of mercury in fish tissue

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Fish consumption is one of the main routes of exposure to mercury in the United States. The Food and Drug Administration (FDA) and the Environmental Protection Agency (EPA) are in the process of revising their 2004 Advisory of Mercury in Fish and Seafood. Current concentrations found in the FDA's monitoring program correspond to values reported as early as 1990 and as late as 2010. The task of updating and maintaining such a database requires the analysis of a large number of samples. Current methods for analysis of mercury in biological tissues require the use of specialized instrumentation such as Cold Vapor Atomic Fluorescence Spectroscopy. For many analytical laboratories this means the acquisition of new instrumentation and training of personnel. Another option is the development of methods that allow the analysis of mercury in biological tissues using instrumentation commonly found in analytical laboratories such as Gas Chromatography (GC). In this project we propose the development of a method for analysis of mercury in fish tissue samples using GC with flame ionization detector (FID). The new method consists of digestion of the fish tissue, phenylation of mercury(II), dispersive liquid-liquid microextraction (DLLME), and analysis by GC-FID.These experiences have given me an insight into the nature of both literature and experimental research.

TP304 Designing a Quality Control Program for Collection and Analysis of Siloxane samples from Environmental Monitoring Programs

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When embarking on an environmental monitoring study, it is critical that the proper quality control (QC) parameters are evaluated to ensure integrity of the results obtained from monitoring activities. This is especially critical when analytes under investigation can be present in personal care products, equipment and analytical instrumentation as is the case for cyclic siloxanes. Selecting an appropriate QC matrix is imperative to accurately account for loss or contamination during collection, transport, and laboratory processing and analysis. In addition, it is important to evaluate analytical variation introduced by the sample matrix itself to the overall signal measured to avoid reporting false positives. It is therefore important that QC matrices be determined prior to the initial design of the monitoring program. Key aspects when designing a QC program include applying an appropriate QC matrix which matches or mimics the sample matrix being collected; determining background contribution from sample collection and laboratory equipment; evaluating initial concentrations present within QC matrices prior to application and how they will evaluate analyte loss or contamination. This presentation will provide an overview of quality control evaluations from several different programs and the best practice for creating a proper QC program. The Loss and contamination of the analyte in the field has been assessed using a variety of materials including fish muscle/liver, sediments and sorbents such as low density polyethylene and XAD® resin. Additionally, processing and analysis QC are assessed to evaluate contamination from equipment such as collection and analysis jars, use of nitrogen as well as analysis equipment.

TP305 Determination of changes in molecular composition of crude oil due to microbial degradation by ultrahigh-resolution FT-ICR mass spectrometry

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The ability of microbes to degrade crude oil in marine systems is well known. However, a detailed understanding of the changes in molecular composition of crude oil undergoing microbial degradation is lacking. To date, changes in oil composition during and after microbial degradation have been assessed using analytical techniques including gas chromatography/mass spectrometry (GC/MS), gas chromatography/flame ionization detection (GC/FID) and Fourier-transform infrared spectroscopy (FTIR). However, GC/FID is unable to discriminate among many compounds in the saturated hydrocarbon fraction of crude oil, and GC/MS cannot measure many high molecular weight compounds. Two-dimensional GC (GCxGC) with MS detection improves resolution, but many compounds in complex crude oil mixtures still cannot be resolved. Newer techniques provide higher resolution measurements that allow simultaneous quantitation of tens of thousands of organic compounds in complex mixtures. One such technique is Fourier Transform Ion Cyclotron Resonance (FT-ICR) mass spectrometry, which can be applied to crude oil to resolve and identify tens of thousands of chemical species at molecular masses measured in the parts per billion range in a single sample. A pilot project was done using a pure strain of Pseudomonas aeruginosa strain WC55previouslyisolated from Apalachicola Bay, FL, capable of using Macondo oil as the sole source of carbon and energy. In this study, a model light, sweet crude oil was incubated with P. aeruginosa in replicate microcosms simulating estuarine conditions over a 40-day period. Changes in molecular composition of oil were characterized using positive and negative ion electrospray FT-ICR mass spectrometry in neutral, acidic, and basic extracts of the degraded oil. This pilot study serves as a precursor to a larger research project to investigate the changes in molecular composition of various types of crude oils (light sweet to heavy sour) during and after microbial degradation by representative in-situ microbial communities present in northern Gulf of Mexico estuarine systems. Our goal is to understand in greater detail the process of microbial degradation of crude oil, and to couple observed changes in oil composition during degradation to specific bacterial communities and metabolic pathways.

TP306 Determination of residual characteristics and risk assessment on dimethomorph, fludioxonil, kresoxim-methyl, and pyridalyl in spinach

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In order to investigate the effect of spraying times on dimethomorph, fludioxonil, kresoxim-methyl, pyridalyl residues in spinach, the test pesticides were diluted 1,000 for dimethomrph (25% WP) and pyridalyl (10% EC), 2,000 for fludioxonil (20% SC) and 3,000 times for kresoxim-methyl (50% WG). And then the diluted solutions were sprayed onto the test crop growing three test plots; twice spraying 7 and 14 days (T-1) or 3 and 7 days (T-2) before harvest and three times spraying 7, 14 and 21 days before harvest (T-3). Residues in the crop were analyzed with an HPLC-DAD for dimethomorph, a GLC-NPD for fludioxonil and kresoxim-methyl, and a GLC-ECD for pyridalyl. Limits of detection (LODs) were 0.004-0.02 mg/kg. Recoveries at two levels, 10 and 50 times of the LODs, ranged from 80.3 to 114.3% in spinach. Amount of the test pesticides residue was highest in T-2, compared with T-1 and T-3. This result indicated that major factor affecting residue level of the pesticide was spraying day close to harvest rather than spraying time. The estimated daily intakes of the test pesticides calculated using the highest residues in spinach were less than 5.2% of their acceptable daily intakes.

TP307 Determining Nitrophenols in Diesel Exhaust Emissions from Heavy-duty Vehicles

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Some nitrophenols, such as 4-nitrophenol, 2-methyl-4-nitrophenol and 3-methyl-4-nitrophenol, have been paid much attention because of their risk of biological effect, such as muscle-contracting and endocrine-disrupting. Diesel exhaust emissions have been known as one of their emission sources. However the detecting method for diesel exhaust emissions has never been authorized. Therefore we have developed a detecting method for not only actual vehicles complying with previous regulations but also vehicles complying with the newest emission regulation in Japan. We will introduce the method and demonstrate the results of emission tests for heavy-duty vehicles by using a chassis dynamometer. The method includes not only filter sampling but also synthetic absorbent sampling because gas sampling by using synthetic absorbents is very important for the vehicles complying with the newest emission regulation. We found most of the nitrophenols leaked through the filter, demonstrating a necessity for a combination of filter sampling and synthetic absorbent sampling to detect the total amount of emissions from the actual vehicles.

TP308 Development of a FT-IR Testing Method for 2,4 D and Dicamba Resistant Soybeans

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Bio-technology companies have developed cultivars of corn, soybean, and cotton with transgenic resistances to the synthetic auxin herbicides; 2,4-dichlorophenoxyacetic acid (2,4 D) and dicamba, which were slated for release in Mississippi for the 2015 and 2016 growing seasons. The introduction of new herbicide tolerant soybeans may provide many benefits for producers such as alternative control options for resistant weed species, decreased costs, and different modes of action. Along with these benefits, the use of auxin containing herbicides may also increase concern for issues such as herbicide drift, volatilization, and tank contamination. To combat these concerns Monsanto in collaboration with BASF (Dicamba-BAPMA) and Dow AgroSciences (2,4 D-choline) have developed new formulations that are less prone to volatilization and drift. Additionally these companies have created product stewardship programs. The Mississippi State Chemical Laboratory (MSCL) analyzes samples each year for off-target deposition of dicamba and 2,4 D, however, current-testing methods cannot differentiate between the amine, ester, or choline formulations. Therefore, our primary objective of this study was to determine the feasibility of using FT-IR technology to identify 2,4 D-choline and dicamba-BAPMA on field samples. Coupling FT-IR spectra to principal component analysis we have been able to separate and identify soybeans that were treated with dicamba-BAPMA and traditional dicamba formulations. The development of additional analytical testing methods will help to ensure an effective stewardship program.

TP309 Direct analysis of systemic insecticides in leaf using SALDI imaging mass spectrometry with sputter-deposited metal film

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In agricultural chemistry, systemic insecticides have been used to administer to plant. Generally, the mobilization of systemic insecticides in plants could be evaluated by high resolution radioisotope imaging,although it is difficult to handle expensive and dangerous radioisotopes. It is necessary for radioisotope experiments to use expensive and exclusive facilities for preventing exposure to radiation. Therefore, simple and inexpensive new methods without the use of radioisotopes for imaging plants are required in agricultural fields to evaluate the mobilization of systemic insecticides and their metabolites in plants. Recently, Matrix-assisted laser desorption/ionization mass spectrometry (MALDI) imaging mass spectrometry (IMS) has been developed for detection of analytes and visualization of the distribution of analytes in a biological tissue. MALDI-IMS of thick plant leaves could not be achieved due to charge-up in the MALDI ion source. The charge-up effect in the ion source during ionization process causes low resolution of obtained mass spectra and low sensitivities of analytes. Therefore, a conductive surface is necessary to obtain good mass spectra in MALDI-IMS. On the other hand, surface-assisted laser desorption/ionization (SALDI) has been investigated for the detection of some analytes which could not be detected by MALDI-MS. I have developed the novel SALDI method. In the SALDI, sputter-deposited platinum film was used as matrix. SALDI with sputter-deposited platinum film (Pt-SALDI) allows IMS analysis of non-conductive materials without the need for extraction because the sputter-deposited film couldimpart conductivity to the surface of samples. In this study, the distribution and mobilization of systemic insecticides in thick and non-conductive leaves was investigated and evaluated. The analytical technique for the direct analysis of distributions and movements of the chemicals in plant leaves using Pt-SALDI-IMS without use of radioisotope are reported. It is conceivable that Pt-SALDI allows the imaging mass analysis of various samples such as plant tissue, biological tissue, paper, and film.

TP310 Dissipation of Pyridalyl and Thiamethoxam Residues in Korean Cabbage and Their Safety Evaluation

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This study was carried out to investigate the residual characteristics and to evaluate safety of pyridalyl and thiamethoxam in Korean cabbage. The test pesticides were sprayed onto the Korean cabbage at 10 days before harvest and samples were collected 7 times, 0 (at 3 hours after spraying), 1, 2, 3, 5, 7 and 10 days after spraying. Limits of detection (LODs) of pyridalyl, thiamethoxam and clothianidin were 0.005, 0.01 and 0.005 mg/kg, respectively. Recoveries of the test pesticides ranged from 74.3 to 104.9, showing that these methods were appropriate for the analysis of the pesticide residues in the crop. Residual amount of pyridalyl from 0 day to 10 days after last spraying on Korean cabbage in field 1 and field 2 ranged from 0.238 to 0.246 mg/kg and from 0.044 to 0.057 mg/kg, respectively. In case of thiamethoxam, the residual amount at day 0 was found to be from 0.06 to 0.08 mg/kg. No residues were found in case of clothianidin, a metabolite of thiamethoxam, and samples sprayed with thiamethoxam at 7 days after treatment. Half-lives of pyridalyl and thiamethoxam residues in Korean cabbage using the equations of their regression curves were found to be from 3.6 to 4.2 days and from 2.2 to 2.7 days, respectively. These results represent that residues of the test pesticides were dissipated gradually after spraying. Also, during the experimental period, dilution effects in residual concentration of the test pesticides by growing of the test crops were negligible considering no significant change in the weight of the crop. The pesticide residues on the harvest day were expected to be lower than their maximum residue limits (MRLs) by predicting with their regression equations. Ratios of estimated daily intakes (EDIs) to acceptable daily intakes (ADIs) of the pesticides (% ADIs) in field 1 and field 2 on the treatment day (0 day) were 1.08 and 1.12% for pyridalyl and 0.64 and 0.85% for thiamethoxam, respectively. Also, those ratios at harvest day and 10 days after spraying were 0.21 and 0.26% for pyridalyl, respectively. These results indicated that the residues of the test pesticides evaluated to be safe.

TP311 Dissipation of residue and safety assessment on cyazofamid, cyhalothrin, and diflubenzuron in perilla leaves

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This study was carried out to investigate residual characteristics of cyazofamid, lambda-cyhalothrin and diflubenzuron in perilla leaves. The test pesticides, cyazofamid (10% SC), lambda-cyhalothrin (1% EC) and diflubenzuron (10% WP) were sprayed three times with 7 day interval onto perilla leaves at a rate of 2,000 for cyazofamid, 1,000 for lambda-cyhalothrin, and 2,500 for diflubenzuron. Samples were sequentially harvested 0, 1, 3, 5, 7 day after last spraying. Limit of quantitation of both cyazofamid and diflubenzuron were 0.02 mg/kg and that of lambda-cyhalothrin was 0.004 mg/kg. Their recoveries ranged from 76.0 and 97.5%, indicating proper analysis methods. Amount of cyazofamid and its metabolite 4-chloro-5-p-tolylimidazole-2-carbonitrile (CCIM) residues were 12.76 mg/kg just after last spraying and 7.92 mg/kg 7 days after last spraying. Also, amounts of the pesticide residues after last spraying and 7 days after final spraying were 1.61 and 0.88 mg/kg in case of lambda-cyhalothrin, and 71.70 and 36.91 mg/kg in case of diflubenzuron, respectively. Residue patterns of the test pesticides including metabolite of cyazofamid were sequently dissipated, showing that half-lives of cyazopamid, lambda-cyhalothrin and diflubenzuron were 10.4, 8.1 and 7.2 days, respectively. Amount of lambda-cyhalothrin in sample was lower than those of cyazofamid and diflubenzuron, representing that not only active ingredient of lambda-cyhalothrin was higher than the others but also lambda-cyhalothrin has non-systemic characteristics. Percent acceptable daily intakes (%ADIs) and theoretical maximum daily intake (TMDI) of the pesticides were below 0.6 and 28.4%, respectively.

TP312 Effect of drying, steaming and boiling on tebuconazole residue in fresh ginseng

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To determine fungicide tebuconazole residues in ginseng and its processed products, such as dried ginseng, red ginseng, and water extracts of dried ginseng and red ginseng, nine trials were conducted from three fields for two consecutive years in Korea. Tebuconazole 20% suspension concentrate combined with 10% trifloxystrobin was applied onto 4-year-old ginseng three times at a nominal rate of 0.134 kg a.i./ha (tebuconazole basis) with a 10-day interval, according to its pre-harvest interval (PHI) in Korea and sprayed consecutively onto 5-year-old ginseng next year. Tebuconazole residues in fresh and processed products were analyzed with GLC-NPD. Average recoveries of tebuconazole in fresh and processed products ranged from 79.40 to 119.48. Reduction factors of dried ginseng, red ginseng, water extract of dried ginseng and red ginseng ranged from 0.37 to 0.41, from 0.34 to 0.37, from 0.19 to 0.20, from 0.16 to 0.35 in first year and from 0.25 to 0.29, from 0.14 to 0.20, from 0.28 to 0.36, from 0.14 to 0.25 in second year, respectively. These results suggested that total amount of tebuconazole residue in fresh ginseng was removed by processing such as oven- or sun-drying, steaming, and high-temperature reflux.

TP313 Effects of Environmental Conditions on Aerobic Degradation of a Commercial Naphthenic Acid

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Naphthenic acids (NAs) are problematic constituents in energy-derived waters (e.g. refinery effluents and oil sands process-affected waters), and aerobic degradation may provide a strategy for mitigating risks to aquatic organisms. The overall objective of this study was to determine the relative influence of four environmental conditions on aerobic degradation of a commercial (Fluka) NA in bench-scale reactors. These conditions included environmentally relevant ranges of nitrogen (N; as ammonia), phosphorus (P; as phosphate), and dissolved oxygen (DO) concentrations, as well as pH and temperature. Commercial NAs provided replicable model compounds necessary to compare influences of environmental conditions on degradation. Changes in NA concentrations for each treatment were measured with time using a derivatization method and high performance liquid chromatography. Microbial diversity and relative abundance were measured and compared among treatments using targeted DNA sequencing via PCR amplification of the v3/v4 region of the 16S rRNA gene. Approximately 50% removal of the initial Fluka NA concentration of 61±8 mg L^{-1} occurred in less than 2 days after NA introduction, achieving the method detection limit (5 mg L^{-1}) by day 6 of the experiment in treatments with a C:N:P ratio of 100:10:1 (in microbial growth medium), DO >8 mg L^{-1}, pH 8-9, and temperatures >23ºC. Negative controls (reverse-osmosis water with NAs and no added nutrients) had no measurable changes in NA concentrations throughout the experimental duration, supporting that NAs were not lost as a result of abiotic factors in treatments. Results indicated that key nutrients, DO, pH, and temperature (within environmentally relevant ranges) influenced rates of aerobic degradation of Fluka NAs. Commercial NAs serve as a simplistic and replicable analog to more compositionally complex mixtures of NAs (e.g. in energy-derived waters). This aerobic degradation study of Fluka NAs could serve as a model for systematically evaluating processes altering exposures of NAs and environmental factors that influence NA degradation in field scenarios.

TP314 Fluorescent assay for cadmium detection based on label-free aptamer

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The selective detection of ultratrace amounts of cadmium ion (Cd2+) is extremely important for food safety and environment since it is one of the most toxic and widespread. In this work, we propose a facile, rapid, excellently sensitive, and highly selective method for the detection of Cd2+, based on label-free aptasensor using unmodified double-strand DNA specific dye (PicoGreen). This system achieved a superior linear dynamic range from 0.1 ng/mL to 100 ug/mL and a detection limit as low as 0.10 ng/mL, which was lower than the toxicity level of Cd2+ in drinking water (3 ng/mL) defined by World Health Organization, and exhibited excellent selectivity to Cd2+ ions. We also tested the application of the aptasensor in real irrigating water samples through spiked with a series of concentrations of Cd2+ and the results showed a good tolerance to matrix effect, compared with atomic absorption spectrometry. The developed approach was a simple, quick and sensitive detection method for Cd2+ monitoring and offered a great potential for on-site and high-throughput analysis in routine control.

TP315 Non-targeted analyses of organic compounds in shortfin mako sharks from US Atlantic waters

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The majority of biomonitoring efforts involve targeted analyses of specific classes of compounds. This approach omits a wide array of potentially harmful chemicals. Recently non-targeted analytical methods have been employed to perform comprehensive screening of environmental compartments for a wide array of Persistent, Bioaccumulative and Toxic species (PBTs). Comprehensive gas chromatography (GCxGC) provides 2 dimensional orthogonal seperation of GC amenable species. Combined with a fast scanning High Resolution Time-of-flight mass spectrometer (GC×GC-HRT, Leco) this technique provides unsurpassed chromatographic and mass spectrometric resolution of environmental matrices, and allows for the chemical fingerprinting of biological tissues. In this study, liver tissue was analyzed from shortfin mako shark (Isurus oxyrhinchus) collected in US waters of the Atlantic Ocean. These highly migratory shark species occupy a high trophic position within the Atlantic and have a global distribution with various known sub-populations. Utilizing the GCxGC-HRT, halogenated and non-halogenated PBTs were detected and cataloged, representing an integrated assessment of the PBTs in the Atlantic Ocean. As expected polychlorinated biphenyls dominated the halogenated profile indicating this legacy contaminant continues to impact marine systems with several polybrominated diphenyl ethers and legacy chlorinated pesticides observed in high abundance. Using NIST, Wiley and in-house spectral libraries 1000's of individual species were identified and the current paper discusses the relative abundance and profiles observed for this novel marine bioindicator.

TP316 Organic pollutants, metals and biochemical biomarkers in Spartina densiflora in estuaries from Argentina, Brazil and Chile

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During the last decades the South- American Atlantic and Pacific coasts have received antropogenic pollutants from industrial, domestic and agricultural sources. As a result, estuarine ecosystems work as sinks of xenobiotics and adverse effects in biota could be expected. Analysis of non- esencial metals, polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCPs) were carried out in sediments and the macrophyte Spartina densiflora. Well-known polluted areas were sellected: Bahia Blanca estuary- Argentina (BB), the mouth of Saco da Mangueira of Lagoa Dos Patos- Brazil (LPII) and Lenga estuary- Chile (LEN). Non or low polluted areas were Mar Chiquita coastal lagoon- Argentina (MCH), the west coast of the Lagoa Dos Patos- Brazil (LPI) and Raqui estuary- Chile (RAQ). In addition, activities of glutathione- S- transferases (GST), catalase (CAT) and guaiacol-peroxidases (POD); total antioxidant capacity (ACAP) and malondialdehide content were measured in roots and leaves of S. densiflora. As it was expected, BB, LPII and LEN showed high levels of pollutants than MCH, LPI and RAQ, respectively. Lead dominated in most of the sediments and plants tissues, with the exception of LEN estuary in which a predominance of mercury was detected. Higher PAHs concentrations were detected in sediments from LPI and roots from LPII. In general, PAHs with 3, 4 and 5 rings predominated in both matrices. The higher total PCBs and OPCs concentrations were detected in LPII followed by BB and LEN. In sediments from Argentina and Brazil endosulfan was the main compound detected while in Chile PCBs and DDTs dominated. Roots from MCH showed the highest concentration of endosulfan, followed by LEN and LPII. Comparing estuarine sediment levels with some Sediment Quality Guidelines (SQG) showed that LEN exceded some effect levels in mercury concentrations mainly. Some clear patterns in biomarkers were observed; lower CAT activity in LPII than in LPI and lower GST activity in BB than in MCH. On the other hand, POD activity and ACAP in roots and leaves were higher in LPII than in LPII. Statistical analysis showed a positive correlation between the concentrations of organic pollutants with POD activity in roots and leaves.

TP317 Quantitative direct injection method and stability study for the detection of sodium fluoroacetate (Compound 1080) and methyl fluoroacetate in water

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To support large scale environmental responses, the United States Environmental Protection Agency (EPA) established the Environmental Response Laboratory Network (ERLN) to serve as a national network of laboratories. The ERLN provides coordinated analytical capabilities and capacities, while ensuring quality data, during a large scale response effort. The EPA's Homeland Security Research Program developed and updates the Selected Analytical Methods for Environmental Remediation and Recovery (SAM) to provide the ERLN with recommended analysis methods for priority chemicals, radioisotopes, pathogens and biotoxins in different environmental matrices. Two priority chemicals, sodium fluoroacetate (Compound 1080, FAA) and methyl fluoroacetate (MFA), lack established, validated methods for water samples. These compounds are toxic vertebrate poisons that could contaminate water supplies and cause widespread harm. This research presents a direct injection liquid-chromatography tandem mass spectrometry (LC-MS/MS) method for analysis of FAA and MFA in water. The detection limits are compared to toxicity threshold values to ensure protection of human health and the environment during an incident. Several water sources will be used to evaluate matrix effects, method interferences, and investigate compound stability in water. The developed method will utilize equipment that is widely available, and possess high throughput capabilities, to assist with lab capacity and capabilitiy issues that arise during large scale remediation incidents.

TP318 Residual characteristics of spirotetramat and its metabolites in dried Angelica gigas Nakai

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This study was carried out to determine residual characteristics of spirotetramat and its metabolites, BYI08330-enol, BYI08330-ketohydroxy, BYI08330-monohydroxy, and BYI08330-enol-glucoside in Angelica gigas Nakai with UPLC-MS/MS. Commercial spirotetramat (22% SC) which was diluted 2,000 times was sprayed onto the crop two or three times. Samples harvested were dried for 10 days under sunlight and then done again with hot-air oven at 50? for 60 hours. Recovery of the residues in the crop ranged from 72.7% to 108.0%. Amounts of spirotetramat, BYI08330-enol, and BYI08330-monohydroxy were less than their LOQ (0.01 mg/kg) in the dried crop. BYI08330-ketohydroxy, metabolite of BYI08330-enol, found to be from 0.02 to 0.10 mg/kg. Also, BYI08330-enol-glucoside, another metabolite of spirotetramat, found to be 0.02-0.06 mg/kg in the dried crop. Total amount of spirotetramat including its metabolires ranged from 0.04 to 0.21 mg/kg in dried crop sprayed two or three times.

TP319 Screening for Perfluoroalkyl Substances (PFASs) in Wildlife Using a Highly Sensitive LC-QTof MS

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Perfluoroalkyl substances (PFASs) encompass a range of fully fluorinated alkyl compounds, typically with an anionic end group. These compounds have been implemented in a range of consumer goods and industrial processes due to their hydro- and lipophobic properties. As a result of their widespread use and subsequent leaching from materials, they have been found in various environmental and biological samples. For monitoring and research purposes, sub-ppb detection of these compounds is often required, in particular for water analysis. Traditionally, this type of analysis has been performed using the selective MRM approach on a tandem quadrupole MS. However, the ability to look for other contaminants of concern after the time of analysis or matrix components such as co-extracted bile acids supports the use of QTof MS. For 11 carboxylic and sulfonic acid PFASs, instrumental performance with respect to determination of limits of detection (LOD; peak-to-peak S/N 1:3) and quantification (LOQ; peak-to-peak S/N 1:10) and linear dynamic range were carried out with solvent standards. Exact mass fragment ion information was also obtained, where at least two ions were observed for each compound. As a result of full spectral acquisition, the entirety of the fragmentation pathways of analytes of interest can be obtained. Diluted mink liver extracts were also analyzed using this method. Samples were prepared with minor modifications according to Kärrman et al. Various PFASs including PFHpS, PFHxS, PFBS, PFOS, PFDS and PFNA in detected levels ranging from 0.2-0.8 ng/mL (without dilution and sample mass correction). In addition to isolating the PFASs of interest, the extraction method also will result in the co-extraction of the bile acid taurodeoxcyholate (TDCA), which co-elutes chromatographically with PFOS. This can result in a very large peak in the analysis, but using exact mass measurements afforded by Tof MS, can be easily distinguished from PFOS.

TP320 Strategic planning of studies to evaluate controls on mercury bioavailability and use of in-situ amendments in tidal marshes of Berry's Creek, NJ

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Berry's Creek (New Jersey, USA) is an urbanized tidal tributary to the Hackensack River which is, in many ways, characteristic of urbanized tidal creek-marsh systems dominated by Phragmites. The detrital food web supports mummichogs and white perch, and the bird community includes sandpipers, red-winged blackbirds, and herons. Studies by university researchers and others from the 1980s through early 2000s observed that mercury (Hg) bioavailability (i.e., net methylation, bioaccumulation) is limited despite quite elevated concentrations of inorganic Hg in Berry's Creek sediments. Beginning in the mid-2000s, we initiated a series of question-driven studies to better understand factors controlling mercury geochemistry, bioavailability, and bioaccumulation in Berry's Creek marshes. In parallel, we conducted studies to evaluate the potential efficacy of in-situ carbon-based sediment amendments to reduce risks in these marshes. These studies were completed as part of an RI/FS under Superfund and involved collaborative participation by academic, private sector, and government scientists. The basic science underlying this work was driven by "top-down" questions intended to help inform risk-based natural resource management decisions. Parallel and sequential studies were carried out in the laboratory and marsh plots to investigate relationships between marsh soil redox and Hg speciation, ligand binding, net methylation, sediment/porewater partitioning of Hg and methyl mercury, biouptake, and the effect of activated carbon on partitioning and Hg biouptake. This presentation focuses on the thought process, healthy debate, and iterative and adaptive learning that went into the planning and implementation of these studies, which collectively help to answer the question Why is mercury bioavailability and bioaccumulation limited in tidal marshes of Berry's Creek, New Jersey?.

TP321 Targeted and Non-Targeted Analysis in a Single Data Acquisition using a Novel Benchtop GC-ToFMS

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As the number and volume of synthetic organic chemicals produced in commerce increases, so does the demand for a practical means to chemically characterize the components in environmental matrices. Non-target analysis provides a broader scope of chemical characterization than targeted analysis, however, targeted methods are still required for quantitation to determine compliance with regulatory guidelines. Here, we present the utility of a new benchtop gas chromatography – time of flight mass spectrometer (GC-ToFMS) to perform concurrent target and non-target analysis on the same sample for a variety of environmental applications including pesticides, EPA 8270, and disinfection byproducts in drinking water. Since GC-ToFMS can be used to acquire full mass range data with every acquisition, with minimal spectral skewing and without a reduction in sensitivity, targeted masses can be selected from the full mass spectra for quantitation, while at the same time full mass spectra can be used to library match non-targeted spectra for qualitative analysis. This enables the user to perform retrospective analysis of archived data to track historic trends and identify potentially new chemicals of concern in environmental matrices.

TP322 Temporal and spatial trends in DDx/DDT ratios in nestling bald eagle plasma from Michigan, USA from 1999-2016

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Bald eagles are top-level predators and important sentinel species for exposure to organochlorine pesticides including dichlorodiphenyltrichloroethane (DDT) and its main metabolite dichlorodiphenyldichloroethylene (DDE). DDT and DDE have been measured in nestling bald eagle plasma from Michigan using solid phase micro extraction and gas chromatography. Plasma samples were collected fron 1999 through the 2016 field seasons ranging throughout the state of Michigan. The five congeners analyzed are 4,4'-DDT, 4,4'-DDE, 4,4'-dichlorodiphenyldichloroethane (DDD), 2, 4'-DDT, and 2,4'-DDE. 4,4'-DDE comprised the majority of total DDT in all samples, ranging from 78-100%. The areas from which the samples were collected were divided according to areas on one of the Great Lakes vs. inland areas, the Upper Peninsula vs. Lower Peninsula, what lake they were nearest, and finally what drainage basin they were associated with if they were inland. Preliminary results show that plasma concentrations from inland birds are lower (9.43 ul/mL) than birds associated with one of the Great Lakes (21.96 ug/mL). Further analysis will focus on 5-year and annual trends in total DDT and DDx/DDT ratios across regions. Additionally, important biological factors such as proximity to an anadromous fish source will be taken into consideration. These data will indicate localized rates of metabolism and the exposure of bald eagles to the DDT and the important metabolites.

TP323 The uptake of several short and long-chain Perfluorinated compounds (PFCs) in foodstuff crops

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Perfluorinated compounds (PFCs) are incredibly stable environmental contaminants. In 2014, the United States Environmental Protection Agency labeled perfluorooctanoic acid and perfluorooctanesulfonic acid, the most persistent and toxic PFCs, as emerging contaminants due to their persistence in the environment. Data from animal studies on perfluorinated compounds indicate that they can cause several types of tumors, neonatal death, and may have toxic effects on the immune, liver, and endocrine systems. While many studies have evaluated the fate of PFCs in aquatic environments, the objective of this study was to determine PFC levels taken up by vegetation under a maximum availability scenario for a terrestrial environment. We grew carrots (Daucus carota), radishes (Raphanus sativus), and wheat (Triticum aestivum) in laboratory grade sand. Sand was chosen due to its low organic carbon content making PFC bioavailability highest. Plant specimens were grown in 200 g of sand contaminated with 10 ng/g (10 ppb) of either perfluorooctanoic acid (PFOA), perfluorohexanesulfonic acid (PFHxS), perfluorobutanesulfonic acid(PFBS), perfluorononanoic acid, or perfluoroheptanoic acid; five PFCs that are currently or previously used in manufacturing and vary in chemical chain length. The plants were watered as needed with nutrient solution and allowed to mature (roughly 60 d). PFC concentrations in plants were determined by LC-MS in an effort to calculate root, stem, leaf, and seed/fruit bioconcentration factors under this scenario. Our pilot study found PFOA whole plant bioconcentration factors ($[PFC]_{plant}/[PFC]_{soil}$) to be around 8 for carrots, 28 for radishes, and 20 for wheat. Bioconcentration factors for PFHxS were approximately 11 for carrots, 58 for radishes, and 35 for wheat. PFBS bioconcentration factors were approximately 54 for carrots, 65 for radishes, and 233 for wheat.

TP324 Trace metal accumulation in tissues of Tilapia nilotica and its relation to calculated free metal ions

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Heavy metal pollution in rivers has become a matter of great concern, not only because of the threat to public water supplies but also due to damage to fishery resources for human consumption. Metals such as Fe, Cu, Zn are essential for its important role in biological systems, while Pb and Cd are not essential, and even toxic at trace levels. Studies on bioaccumulation of contaminants in fish are important to determine the tolerance of fish species, the effects of specific contaminants in fish and bio magnification through the food chain. Bioaccumulation differs even in the same fish species and is given by external influences such as sampling location, season, preferences in diet and body size. Likewise, different organs accumulate metals at different levels. The liver and gills are metabolically active organs, which are targets for the accumulation of metals, while accumulation in muscle tissue is smaller. Great efforts have been made to predict and explain these metal accumulation; nevertheless this has not been accomplished. The present work attempts to correlate the metal accumulation in Tilapia nilotica in a fresh water system with high metal concentration and high organic carbon content. WHAM calculations were made for free metal concentrations in the studied system and correlated to the organism's metal accumulation. Also, PCA analysis was made to associate the metal pattern accumulation.

TP325 Trend of PBDEs in Northern California Pregnant Women

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Polybrominated diphenyl ethers (PBDEs) are components of flame retardant mixtures, which are used in a variety of products including textiles, furniture, and building materials; technical mixtures pentaBDE and octaBDE were banned in 2004 and decaBDE was voluntarily phased out in 2013. PBDEs have been found to be hormone disruptors, especially of estrogen and thyroid hormones, which can be particularly problematic during fetal development. Thus, this study focuses on how phase-out of these technical mixtures has affected PBDE levels found in second trimester pregnant women and their developing fetus(s). Three matrix types were investigated: fetal liver, placenta, and maternal blood serum. Sample collection occurred in 2015-16. To facilitate statistical comparison, women were of similar demographics to the previous studies conducted in 2008-9, 2011-12, and 2013-2014. The liver and placenta samples were analyzed for PBDEs using manual liquid-liquid extraction, while the serum samples were prepared via solid phase extraction utilizing RapidTrace® Solid Phase Extraction (SPE) Workstation. All extracts were quantitated using isotope dilution on a gas chromatograph coupled with high resolution mass spectrometer (GC-HRMS). A previous phase of this study reported a significant drop (39%) in PBDEs (sum of BDE-28, -47, -99, -100, and -153) between 2008-9 and 2011-12. We will investigate whether that trend has continued or has leveled off as observed with past persistent organic pollutants. Results from this study will add to collective biomonitoring data and help track the long term behavior of these pollutants in the human body and environment.

Climate Change and Water Resource Management: An Ever-Changing Challenge

WP001 Freshwater Rhodophyta and Climate Change: Assessment of photosynthetic performance by the chlorophyll a fluorescence method

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Over the last decades there has been a growing concern on evaluating the human influence on Earth's climate. Considering that lotic ecosystems will also suffer effects from anthropogenic climate change, studies on the impact of temperature rise related to Climate Change on lotic primary producers are necessary to have a broader understanding of such effects in these ecosystems. Therefore, we evaluated the effects of temperature increase predicted by two scenarios (RCP 4.5 and RCP 8.5) from the Intergovernmental Panel on Climate Change (IPCC) on the photosynthetic performance of the macroalgae Sirodotia delicatula (Rhodophyta) and its respective "Chantransia" stage, which have been described as important primary producers in shaded tropical streams. We evaluated the photosynthetic performance by chlorophyll a fluorescence technique. Control treatment temperatures for summer (23ºC) and winter (20.8ºC) were determined based on direct measurements of streams from which algal samples were collected. Experimental temperatures were calculated based on seasons control temperatures and the projections proposed by the IPCC. Thus, the summer experimental temperatures defined for RCP 4.5 and RCP 8.5 treatments were, respectively, 25.3ºC and 27.4ºC, while the winter experimental temperatures were of 23.1ºC and 25.2ºC, respectively. S. delicatula showed that the projected increment in temperatures in both IPCC scenarios resulted in no significant change in photosynthetic yields (YII), with only a slight negative variation in nominal values for experimental temperatures in relation to control for both summer (RCP 4.5: -0.48% and RCP 8.5: -4,72%) and winter seasons (RCP 4.5: -15,04% and RCP 8.5: -15,44%). Despite few effects on YII, the RCP 8.5 summer treatment showed a significant increase in non-regulated energy loss (Y(NO)), suggesting that this scenario could cause damage in the photosynthetic apparatus. Although YII of "Chantransia" stage had showed narrow variation in all treatments of both IPCC scenarios, a very significant reduction in YII was observed under RCP 8.5 in summer (-95% in relation to control), indicating that this macroalgae could lose its photosynthetic efficiency in this temperature. From an ecological view, the results showed that the worst IPCC scenario tested could jeopardize the productivity of S. delicatula, both gametophyte and "Chantransia" stage, leading to a reduction in the autochthonous input of energy by this species in tropical streams.

Fate, Toxicology, or Risk Assessment of Materials of Interest to the Military

WP002 Risk Management of Perchlorate Is Influenced by Surface Water Properties That Mediate Amphibian Toxic Response

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As part of the investigations at the former Naval Weapons Industrial Reserve Plant (NWIRP) near McGregor, Texas and nearby offsite properties, an ecological risk assessment evaluated the risk from historical site contaminants to multiple species. Amphibians were identified as a critical species, and a literature-based screening concentration of 0.06 mg/L perchlorate was identified for surface water. Suitable breeding habitats were identified at and in the vicinity of NWIRP McGregor, so sublethal toxicity testing of Spea multiplicata, a species native to the region, was performed to determine the No Observed Adverse Effect Concentration in surface water. Using surface water collected from the vicinity of NWIRP McGregor and perchlorate concentrations of 0.06, 0.11, and 1 mg/L, no effect was observed (Brausch et al, Chemosphere, 2010). Follow-up toxicity testing using Xenopus laevis confirmed the mediating effects of the surface water. Concentrations above 1 mg/L may be more important when attempting to optimize remedial treatment systems. Native species, surface water collected from the vicinity of NWIRP McGregor, and perchlorate concentrations of 1.2, 1.7, 3.0, 5.1, and 8.0 mg/L are being tested to bound the initial study that will be used in optimizing the remediation system and in evaluating future surface water results.

WP003 Comparative toxicogenomics study on the three insensitive munitions constituents DNAN, NQ and NTO in the soil nematode Caenorhabditis elegans

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Ecotoxicological studies on the insensitive munitions IMX-101 and its components 2,4-dinitroanisole (DNAN), nitroguanidine (NQ) and nitrotriazolone (NTO) in various organisms (e.g., fathead minnow Pimephales promelas, the earthworm Eisenia fetida and the freshwater amphipod Hyalella azteca) showed that DNAN was the main contributor to the overall toxicity of IMX-101 and the three compounds might act independently. These results motivated this toxicogenomics study that aimed to discern toxicological mechanisms for these compounds at the molecular level. Here we used the soil nematode Caenorhabditis elegans, a well-characterized genomics model organism, as the test organism and a species-specific, genome-wide 44K-oligo probe microarray for gene expression analysis. In addition to the control treatment, C. elegans were exposed for 24 hours to 6 concentrations of DNAN (1.9-62 ppm) and NQ (83-2667 ppm) or 5 concentrations of NTO (187-3000 ppm) with ten replicates per treatment. The nematodes were moved to a clean environment after exposure. Reproduction endpoints (egg, larvae and offspring counts) were measured at three time points (i.e., 24-, 48- and 72-hr). Gene expression profiling was performed immediately after the 24-hr exposure for each chemical at the lowest, medium and highest concentrations plus the control with four replicates per treatment. Statistical data analysis suggested that chemical treatment did not significantly affect nematode reproduction but did induce 378, 2175 and 118 differentially expressed genes (DEGs) in DNAN-, NQ- and NTO-treated nematodes, respectively. Bioinformatic analysis indicated that the three compounds shared very few DEGs and Reactome-curated biological pathways. Gene set enrichment analysis further demonstrated that DNAN, NQ and NTO significantly altered 20, 84 and 7 unique pathways, separately. Despite the absence of significant reproduction effects, this study provided supporting evidence that the three chemicals, if mixed (e.g., in IMX-101), may exert additive toxicity by independently acting on distinct molecular targets and pathways. Our research findings may be extrapolated to other environmentally relevant species and hence have important implications on environmental risk assessment of materials of military interests.

WP004 Biotransformation of the insensitive munition 5-nitro-1,2,4-triazol-3-one (NTO) in soil

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Insensitive munitions, such as 5-nitro-1,2,4-triazol-3-one (NTO), are being considered by the U.S. Army as replacements for conventional explosives due to their improved ability to withstand unintended detonations. Environmental emissions of NTO are expected to increase as the use of this new class of explosives becomes widespread. However, the fate of NTO in the environment is not well understood. In this study, the transformation of NTO in soil was investigated in batch bioassays under different redox conditions (i.e., anaerobic, aerobic and sequential anaerobic-aerobic). Under anaerobic conditions, NTO undergoes a microbial reduction to 3-amino-1,2,4-triazol-5-one (ATO) when utilizing pyruvate as a electron donor. However, ATO biodegradation was only observed after the redox condition was switched to aerobic. Abiotic controls using sterilized soil demonstrated that NTO persisted in the environment in the absence of microorganisms, indicating that biological activity plays an important role on NTO transformation in soil. ATO-degrading enrichment cultures were derived from an initial culture containing soil, mineral medium and ATO. After multiple transfers (10% v/v), ATO mineralization was investigated through measurements of CO_2 in the headspace and inorganic nitrogen ions in the liquid phase. The results indicate extensive mineralization of ATO, since approximately 100% of CO_2 and approximately 50% conversion of nitrogen to inorganic ionic N species were observed. The results obtained in this research suggest that NTO mineralization can be achieved through a sequential anaerobic-aerobic approach, decreasing the risks of this compound to the ecosystem and to public health.

WP005 Dissolution of insensitive munition constituents and their fate and transport in soils

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Insensitive munitions (IM) were developed to prevent unintended detonations. 2,4-dinitroanisole (DNAN) and 3-nitro-1,2,4-triazol-5-one (NTO) are two new energetics which together with nitroguanidine (NQ), and hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) are used in IM formulations IMX-101 and IMX-104. Quantifying both their release from the formulation particles and their further environmental fate is critical for estimating risk for humans and ecosystems. We are presenting updates from our project where dissolution of IM particles and change in their structure was measured in the lab; their dissolution and phototransformation under different climatic conditions quantified, and products characterized; and adsorption measured for a wide array of soils. We also estimated transport of these compounds in soils by adding aqueous solutions or IM particles to the soils and measuring patterns of release and transport. We observed that IM constituents dissolved sequentially as predicted by solubility, first NTO, followed by NQ, and DNAN in IMX-101, and DNAN and RDX in IMX-104, a sequence that can be expected under field conditions. Fast removal of the soluble components resulted in porous particles that crumbled, further enhancing dissolution. About 65% of particle mass was dissolved in a little more than two years. The change in color of the particles exposed to sunlight and the detection of compounds not present in formulations indicated the occurrence of phototransformation. Due to the faster dissolution of IM formulations only about 19% of mass loss could be attributed to phototransformation, less than for traditional explosives. As NTO adsorption in soils was low when particles were dissolving very high peak concentrations of NTO were observed traveling through soils with little to no retardation. With time concentrations decreased and continued at a relatively constant rate. A similar pattern but lower concentrations were observed for NQ, while DNAN and RDX had lower concentrations in leachates that did not change over time and were delayed relative to NTO and NQ due to soil adsorption. The fast dissolution of NTO and its low attenuation in soils indicate high risk of its transport to the ground and surface waters, whereas DNAN slower dissolution and soil adsorption indicate lower potential for off-site transport.

WP006 Wash-off Coefficients of Chemical Warfare Agent (VX) from Foliar Surfaces of Living Plants

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Defensive capabilities are needed to identify threats in the event of the release of Chemical Warfare Agent (CWA) into the natural environment. A CWA disseminated in the field can follow many pathways that affect its fate, which may ultimately result in exposure hazards. An important exposure pathway is the CWA–plant interface. Persistence, penetration (absorption), or evaporation of agent droplets on foliage depends on many factors including vapor pressure, the hydrophilic nature of the CWA, and the extent of hydrophobicity of foliar surfaces. Our objectives in the present study are to determine if rainfall reduces the exposure hazard to the warfighter by decreasing the amount of CWA available on the leaf surface for transfer by contact, and provide data for predictive models to estimate the environmental fate of CWA and related chemicals. We experimentally established wash-off coefficients (kw) for the CWA O-Ethyl-S-(2-diisoproylaminoethyl) methyl phosphonothiolate (VX) on grass (Echinochloa crus-galli), in separate replicated experiments utilizing 1 and 3 µL VX droplets, at 0.017 (1min), 1, and 4h post-dissemination. A 10mm (0.39”) rain event at 0.017h post-dissemination washed off 95% (1 µL droplets) and 83% (3 µL droplets) of the VX, respectively. However, VX readily absorbed into leaves, and at 1h post-dissemination, a 10mm rain event washed off 0.03 and 0.5%, respectively, of 1 and 3 µL VX droplets. The kw values for 1 and 3 µL droplets, respectively, at 0.017h post-dissemination are 0.095 and 0.083 mm-1. At 1 and 4h post-dissemination, kw values were approximately 3 orders of magnitude less than those at 0.017h. We also exposed grass contaminated with 3µL VX droplets to multiple (10x) light rain events (100µL) followed by multiple (10x) surface wipes, at 0.017, 0.5, 1, 4, and 24h post-dissemination. The cumulative proportions of 3 µL VX droplets washed off at 0.017 and 1h post-dissemination were 75.3 and 0.99%, respectively, whereas the respective cumulative proportions of VX that were wiped off following those rain events were 0.8 and 0.3%. Based on these results, the majority of the VX on grass (approx. 75-95%) can be removed by wash-off from a moderate rainfall (≥0.6mm), or with excess water, within minutes post-dissemination of VX; however, the hazard is then transferred to the water and soil. Results from these investigations provide functional information for Go/No Go decisions affecting Soldiers on CWA-contaminated battlefields.

WP007 Laboratory Hood Culture of Terrestrial Plants for Investigations Involving Highly Toxic Materials

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Field investigations suffer from an inability to adequately control inherently unstable ambient variables. Standardized plant tests conducted under controlled environmental conditions allow investigation to focus on factors that directly affect critical parameters. However, it is necessary to maintain normal plant physiological responses in order to obtain results applicable to the field. We successfully sustained healthy living plants within laboratory hood constraints, and safely disseminated highly toxic compounds onto individual mature leaves of intact living plants. Traditional plant culture in growth chambers or greenhouses typically involves balancing heat loads with large chilling units too cumbersome for most laboratory hoods. Recent technological advances in blue light-emitting diodes (LED) now permit delivery of high-quality photosynthetically active radiation (PAR) in sufficient quantity to maintain plant physiology and sustain extended culture of healthy terrestrial plants. We installed and tested a lighting system consisting of improved LEDs to provide PAR in a laboratory surety hood. Environmental conditions in the hood were: PAR illumination >300 umoles cm^2 sec^{-1}, 16h-light/8h-dark;

22°C ±2; relative humidity >50% ±10; air flow 1.5mph ±0.09. We selected the grass E. crus-galli as the plant for method development and subsequent research. Grass is the most prevalent higher plant worldwide, and the natural distribution of E. crus-galli is one of the largest. We constructed stands with adjustable rings to hold plant containers in a fixed position, with petri dishes below each container for consistent bottom-up irrigation. Individual leaves were secured in a horizontal position onto rings by lengths of cellulose acetate tape folded onto itself, then placed across the leaf surface, thus preventing sticky contact, and tape ends were then secured. This prevented leaf damage by tape removal, yet ensured that the disseminated compound contacted the leaf surface at the points intended, and identified those locations for further investigation. Critical parameters successfully established include: characterization of droplet-spread, distribution within leaves as a function of time, coefficient of wash-off from measured rainfall, Effective Half-Life, Contact Transfer (exposure) from contaminated foliage. Results comport with those from field studies, thus allow standardized comparisons among compounds, plant effects, and respective corresponding hazards.

The Role of Stressors for Species Interactions and Food Webs

WP008 Stress response in round stingrays exposed to environmental PCBs along the southern California coast

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Stress response impairment due to environmental contaminant exposure has been documented in a variety of organisms; however, little research has investigated these types of effects in elasmobranch fishes. Round stingrays (Urobatis halleri) sampled from mainland California (Long Beach) had significantly higher hepatic PCB levels than stingrays from a nearby offshore island (Catalina). We tested the hypothesis that higher PCBs body burden will compromise the stress response in this species. Stingrays were collected via hook and line from these two locations and plasma was sampled either immediately or 15 min after a confinement stressor. Our results support the hypothesis that Catalina rays showed a corticosteroid response, while this conserved response was attenuated in the mainland rays. Also, liver glucose and glycogen content were lower in the stressed mainland stingrays compared to the Catalina rays. Overall, PCB contamination reduces the capacity to elicit a physiological stress response in the round stingrays. This study was supported by NSERC, Canada, Discovery Grant to MMV and a Vanier PhD Scholarship to KL.

WP009 Influence of Manatees' Diving on Their Risk of Collision with Watercraft

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Collisions with watercraft account for approximately one quarter of Florida manatee (Trichechus manatus latirostris) deaths annually. Reducing the effects of this environmental stressor on this endangered subspecies is an important management goal. To assess risk factors that contribute to watercraft-related injury to manatees, we studied the dive behavior of nine manatees carrying GPS tags and time–depth recorders in Tampa Bay, Florida, during winters 2002–2006. We applied a Bayesian formulation of generalized linear mixed models to depth data to model the probability that manatees would be no deeper than 1.25 m from the water's surface as a function of behavioral and habitat covariates. Manatees above this threshold were considered to be within striking depth of a watercraft. Roughly three quarters of the depth records were within striking depth and strike risk was higher in shallow water, over seagrass, at night, or while stationary or slow-moving. They were less likely to be within striking depth when located 50 meters or less from a charted waterway. The probability of a manatee being within striking depth decreased as water depth increased. Strike probability was greater when the manatees were located within seagrass beds. Finally, in some circumstances, manatees made consecutive dives to the bottom while traveling, even in areas >14 m, possibly to conserve energy. This is the first documentation of potential cost-efficient diving behavior in manatees.

WP010 Monitoring changes in fish community composition relative to the occurrence of an extended harmful algal bloom/seagrass die-off event

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A series of massive phytoplankton blooms (superbloom) beginning in early 2011 triggered habitat changes, most notably a loss of seagrass, throughout much of the Indian River Lagoon, Florida (IRL). The superbloom and subsequent habitat loss prompted a multi-agency effort to investigate and monitor the impacts of the resultant changes to the IRL ecosystem. The superbloom, which extended from southern Mosquito Lagoon to Ft. Pierce Inlet, had a significant impact on seagrass beds in subsequent years (up to 100% loss in some transects) in all three IRL sub-basins. Comprehensive and standardized fisheries data were used to compare with conditions prior to the superbloom as well as to analyze trends in relative fish abundance. In general, the observed decline in drift algal habitat, which was below the long-term average during the superbloom, has improved in post-bloom years. Fish health issues (e.g. lesions, parasites) increased during and after the superbloom. There were significant differences in community metrics among the pre-bloom, bloom, and post-bloom years. Relative abundance of most small-bodied/cryptic species and juveniles of several sciaenids (drums, croakers, etc.) declined during the bloom year. In contrast, sub-adult and adult relative abundance of these sciaenid species increased during the bloom year. A mixed response from certain planktivorous taxa was observed. Juveniles of some taxa (e.g., Spotted Seatrout, Pinfish) may have compensated for the loss of seagrass and drift algae habitat by utilizing tributaries and other alternate habitats in affected areas.

WP011 Bioaccumulation and trophic transfer of permethrin in pyrethroid-resistant Hyalella azteca

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Some field populations of the freshwater amphipod, Hyalella azteca, demonstrate resistance upon exposure to pyrethroid insecticides. Standard toxicity tests show that a resistant population is 53 times less sensitive to the pyrethroid, permethrin, than a non-resistant population, and that the resistance to permethrin is stable even after 16 months of pyrethroid-free culturing. One of the possible consequences of resistance is increased bioaccumulation, which increases the potential for transfer of pyrethroids from the resistant individuals to higher trophic level organisms. In the current study, resistant and non-resistant H. azteca were exposed to ^{14}C-labeled permethrin, and bioaccumulation and biotransformation were quantified. At lower permethrin exposure concentrations, the tissue concentrations and proportion of parent permethrin in resistant and non-resistant H. azteca were similar. As the exposure concentration was increased, the non-resistant H. azteca did not survive, while resistant H. azteca bioaccumulation increased with increasing permethrin concentration. The trophic transfer potential of permethrin via resistant H. azteca was demonstrated by daily feeding of permethrin-exposed H. azteca to fathead minnows (Pimephales promelas). After four days of feeding, the tissue concentrations in the fish reached 476 ng total permethrin/g lipid. Because resistant H. azteca can survive elevated permethrin concentrations, this subsequently increased the risk of permethrin bioaccumulation to fish through trophic transfer. The proportion of parent permethrin decreased from 47% in H. azteca to 33% in the fish tissue, which suggested that the acute toxicity to fish due permethrin is low. However, permethrin and its biotransformation products have sublethal toxic effects on some fish species. These results suggest that pyrethroid-resistance in H. azteca populations may increase fish exposure to permethrin and its biotransformation products, which may impact wild fish populations.

WP012 Mercury Contamination and Thermal Stress Combines to Have Detrimental Effects on Freshwater Mussel-Driven Ecosystems

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Modern freshwater ecosystems face an ever-growing array of threats and stressors. Among the most widespread are mercury (Hg) contamination, impoundment and flow alterations, and global climate change (GCC). Hg emitted from anthropogenic sources is converted into toxic methylmercury in aquatic ecosystems where it biomagnifies in the foodweb, threatening wildlife and humans, while impoundment, reduced in-stream flow, and GCC can contribute to increased thermal stress in many freshwater ecosystems. We conducted two experiments to test the combined effects of Hg and thermal stress modeled after the mussel-driven ecosystems of southeastern Oklahoma. A laboratory experiment examining the effects of Hg and thermal stress alone and in combination found decreased respiration and clearance rates in some mussel species and high mortality in a thermally sensitive species when exposed to both stressors. This study was followed-up with a mesocosm experiment examining the effects both stressors on communities with and without mussels. Increased mussel mortality was observed again in double stressor treatments for multiple species. Because freshwater mussels contribute significantly to the diversity and functioning of numerous rivers and streams across North America (including food web structure) these findings have important ramifications for management and conservation of these already stressed and imperiled ecosystems.

Toxicity Extrapolations in Aquatic Organisms and Wildlife

WP013 Toxicological estimation of mortality of oceanic sea turtles oiled during the Deepwater Horizon oil spill

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During the Deepwater Horizon (DWH) oil spill hundreds of externally and internally oiled juvenile oceanic sea turtles were documented and rescued offshore and sent to rehabilitation facilities for treatment and observation. However, the entire Gulf of Mexico was not surveyed and the search effort for exposed turtles was limited to a period covering a few weeks after the spill and before the wellhead was capped. Therefore, the number of sea turtles dying in the wild during the spill or afterward is unknown. As part of the NRDA effort we employed multiple lines of evidence to estimate the mortality of oceanic sea turtles that were minimally to moderately oiled by the 2010 BP Deepwater Horizon (DWH) oil spill in the Gulf of Mexico (GoM). This included using estimates of the oil ingested by oceanic sea turtles that were rescued. As there is limited knowledge on the sensitivity of turtles to oil or the effects of oil spills on reptiles, the oil ingestion estimates were compared to toxic endpoints following oil ingestion in turtles and other vertebrate species so as to derived an estimated percentage of mortality for oil-exposed oceanic sea turtles. Oil ingestion in oceanic sea turtles was estimated based on extent of oiling categories assigned by the sea turtle technical working group (STTWG) (i.e. nonoiled (0); minimally oiled (1), lightly oiled (2), moderately oiled (3) and heavily oiled (4)). Because the STTWG concluded that 100% of heavily oiled turtles would have died from the physical effects of heavy oiling, we limited our assessment of mortality to turtles in categories 0-3, and estimated how many of these turtles would have died from ingestion of oil. We estimated mortality at 85% for category 3, 50% for category 2, and 25% for category 1. Visibly unoiled turtles (category 0) were assigned 0% mortality. To calculate the overall mortality for all turtles the mortality estimations for categories 0-3 were applied to the numbers of turtles observed with different degrees of oiling, as documented by direct capture operations during the DWH spill. We concluded that overall, approximately 30% of all oceanic turtles in the region affected by the DWH spill, that were not heavily oiled, would have died from ingestion of oil.

WP014 Modification in sensitivity of microalgae to 3,4-dichloroaniline. Comparison of exposure as single species or as assemblage of different species

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The aromatic amine, 3,4-dichloroaniline (3,4-DCA), is used in the synthesis of azo dyes, bactericides, and herbicides; 3,4-DCA is also a byproduct of biodegradation of herbicides. This amine is very toxic to aquatic biota, being classified as dangerous for the environment. Toxic effects of pollutants in microalgae are frequently assessed in single species tests, but different effects could be expected when algal communities are exposed to toxicants. The aim of this study was to determine if growth inhibition caused by toxics in microalgae changes when algae are exposed collectively including species of different sensitivity. The 96-h median inhibitory concentrations (IC_{50}) for 3,4-DCA were determined in Ankistrodesmus falcatus, Chlorella vulgaris, Pseudokirchneriella subcapitata, and Scenedesmus incrassatulus. After this, 0, 2, 4, and 8 mg L^{-1} 3,4-DCA concentrations were tested in the assemblage of the four microalgal species. Four replicates for each treatment were incubated at 27°C, 83.25 µmol m^{-2} s^{-1} luminous energy, and 120 rpm orbital shaking during 9 days. Daily the cell density of each species was recorded, and for S. incrassatulus the phenotypic plasticity was also documented. The photosynthetic pigments concentration and the main macromolecules were quantified at the end of the assay; SEM and TEM images were also obtained. A. falcatus was the most sensitive species (IC_{50} = 0.38 mg L^{-1}), whereas the most tolerant was S. incrassatulus (IC_{50} = 5.58 mg L^{-1}); P. subcapitata and C. vulgaris showed a similar, intermediate tolerance (IC_{50} = 2.35 and 2.5 mg L^{-1}, respectively). In the algal assemblage (community) experiment, at the end of exposure, C. vulgaris predominated in the control whereas S. incrassatulus was dominant in all the 3,4-DCA concentrations. Toxicant exposure produced morphological changes and altered the structure of cell organelles, as revealed by SEM and TEM analyses. Concentration of photosynthetic pigments, protein, and lipids was also affected significantly. The morphology and number of cells per coenobium in S. incrassatulus was modified, depending on the exposure time and the toxic concentration. The structure of the assembled community provided protection to species and increased their tolerance, as compared to individual exposure. Results demonstrated that assessment of toxic effects of pollutants through single species assays could provide different sensitivity data to those potentially produced in an algal community.

WP015 Toxicity assessment of Congo Red dye using a battery of bioassays (Chlorella vulgaris, Daphnia magna, and zebrafish embryos)

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Azo dyes are widely employed in the textile and paper industries due to their low cost and easy use. Extensive consumption of synthetic dyes produces large volumes of wastewater discharged into aquatic ecosystems. Colored effluents produce toxic effects in the aquatic biota, reduce light penetration in water and alter the photosynthetic activity, causing oxygen deficiency, among other effects. Some dyes, such as Congo Red, are elaborated with benzidine, a known carcinogenic compound. Information regarding dye toxicity in aquatic ecosystems is reduced; therefore, our study was aimed at evaluating the toxic effect of Congo Red on a battery of bioassays: Chlorella vulgaris, Daphnia magna, and Danio rerio. C. vulgaris was exposed to 5, 10, 15, 20 and 25 mg L^{-1} Congo Red, (96 h at 25°C, continuous illumination of 120 µmoles m^{-2} s^{-1}). Daphnid neonates were exposed to 100, 200, 300, 400 and 500 mg L^{-1} Congo Red (48 h at 22°C, light intensity of 120 µmoles m^{-2} s^{-1}, and 16:8 h photoperiod). Zebrafish embryos were exposed to 6.25, 12.5, 25, 50 and 100 mg L^{-1} (96 h at 26 + 1°C, light intensity of 120 µmoles m^{-2} s^{-1},and 16:8 h photoperiod). C. vulgaris was the most sensitive species to Congo Red (IC_{50} = 5.14 mg L^{-1}). The cladoceran D. magna was tolerant to high concentrations of Congo Red, we determined the LC_{50} at non-environmentally relevant concentrations (322.88 mg L^{-1}), but we observed that the digestive tract of test organisms was remarkably red colored, hence toxic effects could be expected at sub-chronic exposures. On the other hand, no lethal effects were observed in Danio rerio embryos at 96 h (according to OECD guideline 236 criteria), but larvae were unable to break the chorion and hatch, even at times as long as 9 days, so they finally died at all tested concentrations. Results demonstrate that Congo Red dye affects diversely organisms of different trophic levels. Particularly, the effects observed in microalgae confirm the vulnerability of primary producers to colored wastewaters because dyes produce toxic effects and interfere with photosynthesis. Obstruction in fish larvae hatching is also a significant consequence that warns about the effects of Congo Red dye in fish. Azo dyes should be considered as emerging pollutants because they are continually discharged into the aquatic environment, and are not currently included in environmental monitoring and regulation programs.

WP016 Exposure to 3,3',4,4',5-pentachlorobiphenyl (PCB 126) Impacts Multiple Organ Systems in Developing Little Skate (Leucoraja erinacea)

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Effects of exposure to coplanar polychlorinated biphenyls (PCBs) and other dioxin-like chemicals on developing vertebrates involve many organ systems, including the skeletal and cardiovascular systems. Apex predators, including those from the class Chondrichthyes (sharks, skates, and rays), accumulate high body burdens of PCBs through biomagnification of chemicals moving through food webs. There are no published reports of the effects of dioxin-like chemicals on the development of sharks, skates, or rays. A study was undertaken to assess developmental effects of 3, 3', 4, 4', 5-pentachlorobiphenyl (PCB 126) exposure in little skate, Leucoraja erinacea, a model for oviparous elasmobranchs. Skate embryos cultured outside of their egg cases were exposed to 0.02 – 20 ng/ml PCB 126 for 6 days and then grown in clean seawater for up to 29 days. Gas chromatography was used to measure PCB 126 in the exposures water and quantify its accumulation in the embryo. Digital still and video imaging was performed to assess growth, identify developmental abnormalities, and cardiovascular function. Embryos accumulated approximately 50% of PCB 126 exposure mass in the embryonic tissues and yolk sac. All embryos in the control and 0.02 ng/ml treatment survived; mortality rates were 14, 52, and 40% of embryos exposed to 0.2, 2.0, and 20.0 ng/ml, respectively. PCB 126 exposure induced yolk sac edema, deformities of the jaw, cranium, and fins, and cardiovascular system failure in skate embryos at all concentrations. This study demonstrates that little skate embryogenesis is sensitive to the toxic effects of PCB 126. Many of the developmental effects of PCB 126 in skate embryos are similar to that observed in other vertebrates, however, the alteration in pectoral fin architecture is novel and may represent disruption of developmental pathways that differ between cartilaginous and bony fish appendages. The research continues to ascertain the no observable effects concentration for PCB 126 and identify sensitive developmental pathways using the little skate model.

WP017 Oxidative stress in the goodeid fish Chapalichthys pardalis induced by exposure to the herbicide glyphosate

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In Mexico 900 pesticides are currently used, distributed over 260 brands, of which 24 are prohibited in other countries. Herbicides are the pesticides most frequently used in Mexico, being glyphosate the most common because it is cheap and easy to use. Glyphosate is normally mixed with different inert ingredients like haulers, antifreezes, antifoams, dyes and surfactants, which sometimes increase toxicity of the active ingredient. Michoacán is the seventh consumer of pesticides in Mexico, and also it is the habitat of the micro endemic goodeid Chapalichthys pardalis. This fish is distributed in a puddle surrounded by agricultural areas in Tocumbo, Mich. Yet the importance of Goodeidae as an endemic family that includes several endangered species, the effects of herbicides on the antioxidant response is poorly known. For this reason, the objective of this study was to determine the toxicity of glyphosate in the commercial formulation named FAENA^{MR}, and as active ingredient, assessing the effect on the oxidative stress levels in C. pardalis. Adults of C. pardalis were exposed to environmentally relevant concentrations of glyphosate both as the commercial formulation and as active ingredient. The concentration of carbohydrates and lipids, the antioxidant enzymatic activity of superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) were determined; as well as oxidative damage measured as TBARS (related to lipid peroxidation), and protein oxidation; these biomarkers were analyzed in different tissues (gills, liver and muscle). Two-way ANOVA and Fisher LSD test for multiple comparisons were used for data analysis. Higher concentration of TBARS in the three organs was observed in the commercial presentation vs. pure glyphosate (gills 1.657 vs. 0.295, liver 1.597 vs. 0.297 and muscle 0.522 vs. 0.111 nM mg^{-1} of tissue), but no significant differences among concentrations were observed. Gills, liver and muscle showed protein oxidation among treatments ($p < 0.001$) and concentrations ($p < 0.001$). High concentrations of glyphosate in the commercial formulation (>180 mg L^{-1}) increase the protein carbonylation (oxidation). Obtained results confirm that the commercial formulation FAENA^{MR} is more toxic than the pure active pesticide. This herbicide provokes oxidative stress by lipid and protein oxidation in different organs of C. pardalis. The present results warn about the damage associated with the use of this herbicide on endemic fish species.

WP018 Exposure to silver nanoparticles produce effects in physiological biomarkers and modifies the antioxidant response in Chapalichthys pardalis

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In recent years, the industry of nanomaterials has become very important in various areas and its applications are still growing in all fields. This has created a great concern about their impacts on the environment. Is expected, that the high production of nanomaterials could increase the levels of its discharges on water bodies, causing adverse effects on the biota. Moreover, it has been documented that metal nanoparticles may represent a potential risk to aquatic organisms since previous studies have reported their capability to cause oxidative stress and morphological alterations in hydrobionts. In Mexico, silver nanoparticles (AgNP) are the most produced and commercialized due to its antibacterial properties; however, no information about its aquatic toxicity, specifically with native species, is available. in this study we evaluated the 96-h acute toxicity of AgNPs (LC_{50}) in adults of Chapalichthys pardalis; after this, subchronic toxicity during 21 days to two sublethal concentrations (equivalent to the LC_1 and LC_{10}) was evaluated through quantification of an array of biomarkers: activity of antioxidant enzymes (SOD, CAT and GPx), lipid peroxidation inferred from the concentration of thiobarbituric acid reactive substances (TBARS), concentration of macromolecules (protein, lipids and carbohydrates), and metabolic parameters (glucose and lactate), on liver, gills and muscle of the goodeid endemic fish Chapalichthys pardalis. The results were analyzed with the Integrated Biomarker Response (IBR). The LC_{50} was 10.32 mg L^{-1}. Results of sub-chronic exposure showed that AgNPs produced oxidative stress in adults of C. pardalis, since decreased activity of antioxidant enzymes, increased levels of TBARS, proteins oxidation, and increased energy consumption were documented. The analysis of IBR evidenced that the greatest effect occurred in organisms exposed to LC_{10}. The gills (IBR = 10.07) and liver (IBR = 5.37) were the tissues that suffered further damage, and the muscle was the less affected (IBR = 2.73). We conclude that the AgNPs are potentially risks to endemic aquatic biota, which could be exposed to xenobiotics because of water pollution.

WP019 Antioxidant and metabolic maternal-embrionary response of Chapalichthys pardalis exposed to 3,4-dichloroaniline

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Goodeinae are endemic viviparous fish distributed in the Mexican Central Plateau. The habitat of these fish has been transformed, mainly as result of chemical pollution, but the effect of chemical stress in wildlife has been poorly documented. All Goodeinae fish have evolved an exceptional reproductive mechanism characterized by intra-ovarian gestation, internal fertilization, matrotrophy and the development of trophotaeniae in embryos. The great maternal-embryonic interaction during gestation could increase the risk to the progeny, when adults are exposed to toxicants. The aim of the present study was to determine the maternal-embryonic antioxidant and metabolic response in C. pardalis exposed to 3,4-dichloroaniline (3,4-DCA), a reference toxicant used for the synthesis of common herbicides, and byproduct of herbicide degradation. The median lethal concentration was determined (LC_{50}); after this, gravid females were exposed to sublethal concentrations of 3,4-DCAduring 21 days. The antioxidant enzymes activity (superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx)) was assessed in different organs of the exposed gravid females, and in the embryos. Concentration of macromolecules (protein, lipids and carbohydrates), glucose, lactate, thiobarbituric acid reactive substances (TBARS), and protein oxidation were also measured. All results were analyzed using the “Integrated Biomarker Response” (IBR) for a whole interpretation of toxicity effects. The LC_{50} was 5.18 mg L^{-1}(confidence limits: 4.8-5.5 mgL^{-1}). All the embryos were aborted ingravid females exposed to 3.3 and 2.5mg L^{-1}. Females exposed to 0.5 mg L^{-1} showed antioxidant activity alterations and oxidative damage, being the most affected the liver of adults and the embryos (IBR of 10.8 and 9, respectively). Macromolecules concentration was significantly affected in embryos and adults’ liver (IBR= 14.36 and 8.44, respectively). We can conclude that maternal exposure to environmental xenobiotics, such as 3,4-DCA,constitutea risk for embryo development and for the survival of C. pardalis.

WP020 Comparison of pharmaceutical and signaling pathway inhibitor effects on expression of genes related to swim bladder inflation in Japanese medaka

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Inadequate swim bladder inflation can have serious long term effects on many larval fish populations. Many different environmental contaminants including pharmaceuticals have been shown to inhibit swim bladder inflation. Understanding how this occurs and developing biomarkers for this effect are thus important. This study first established that embryonic exposure to 5µM cyclopamine, a Hedgehog (Hh) signaling inhibitor, as well as 1µM IWR-1, a wnt signalling inhibitor, resulted in >95% inhibition of swim bladder inflation of Japanese medaka (Oryzias latipes) larvae. The effects of these inhibitors on the expression of medaka genes related to the Hh and wnt pathways, as well as genes involved in the formation of the three cell layers that make up the swim bladder (epithelial, mesenchyme and outer mesothelium) were determined at 80, 96, 101, 144, 180 and 216 hours post fertilization. The effects on expression of key genes related to swim bladder formation and inflation were measured following exposure to 17α-ethinylestradiol, levonorgestrel, and diclofenac to determine if these pharmaceuticals act on similar targets or pathways related to Hh or wnt signaling.

WP021 Effects of Dicofenac on the Expressions of Genes Related to Detoxification System in Mugilogobius Abei

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An increasing number of pharmaceuticals and personal care products (PPCPs) have been found in the aquatic environment and the issue has become a global environmental health concern. Dicofenac is a widely used non-steroidal antiinflammatory drug (NSAID) and is frequently detected in the various aquatic environments, such as wastewater effluents, surface waters, groundwaters and even drinking water due to its relative long half life periods. With the increase of consumption of the pharmaceuticals and subsequent their discharge into the aquatic environment in recent years, they are present at detectable levels in most sewage effluents. Now information available is still not enough about their potential impacts on aquatic organisms, especially on responses of genes related to fish detoxification metabolisms. In this study, real-time quantitative PCR was used to investigate the effects of dicofenac on aquatic fish Mugilogobius Abei. First, we cloned PXR, CYP3A and Alpha-GST of Mugilogobius Abei and established a stable and believable method to test the mRNA and microRNA alterations. The transcriptional expressions of PXR, P-gp,CYP3A and GST as well as the alterations of their corresponding microRNAs (miR-148a, miR-27a, miR-27b, miR-34a, miR-34b and miR-34c) related to detoxification system were determined. The results showed that there existed significant dose-effect relationship between GST, P-gp and dicofenac concentrations. A similar trend between PXR mRNA expression and P-gp, CYP3A and GST genes was observed in the exposure of dicofenac, suggesting PXR-CYP3A, PXR-Pgp and PXR-GST pathway for the DCF detoxification exists in Mugilogobius Abei. No significant changes were observed in all microRNAs tested. However, the genes and microRNAs were more sensitive in the exposure of 5 µg L-1 dicofenac, a negative correlation between miR-148a, miR-27a, miR-27b, miR-34a, miR-34b and miR-34c with P-gp was observed. It is a complex process in the detoxification of xenobiotics, in which nuclear receiptors-regulated and miRNA-mRNA pathways may play an critical role.

Activated Carbon as a Remedial Alternative for Management of Risks in Contaminated Sediments

WP022 Activated Carbon in Sediment Remediation – Experiences from laboratory and field studies

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In-situ treatment of contaminated sediments can be an effective, uncomplicated and less invasive method than traditional approaches, such as dredging. Activated carbon (AC) has quickly become one of the most promising tools for this purpose, as it is capable of binding large amounts of persistent organic contaminants, thus rendering them unavailable for the uptake by organisms or release into the water phase. While the high contaminant binding potential has been shown in both field and laboratory trials, the adverse effects of AC amendments seem to be more pronounced in the latter. For this reason, we are utilizing both approaches and aim at a direct comparison from laboratory-based predictions and observations in the field. The field trial was set up in the PCB contaminated Lake Kernaalanjärvi, Finland. A 300m^2 plot was amended with an AC thin layer cap (1.66 kg AC/m^2) in 2015. The PCB bioaccumulation and the ecological condition of the benthic fauna on the plot and several adjacent reference sites will be monitored. In addition, sediment traps were used to determine the amount, composition and PCB-content of newly deposited sediment. Passive samplers (coated vials and PE strips) were used to determine freely dissolved aqueous concentrations of PCB's. Laboratory studies were executed to predict the effectiveness and risks of AC amendments, including scenarios with ongoing sedimentation of contaminated material (as it is the case on the studied field site). In microcosms, AC thin layer caps were covered with an additional layer of natural, PCB contaminated sediment in varying thicknesses to simulate different sedimentation rates. First results using Chironomus riparius larvae show that PCB bioaccumulation is reduced significantly even if the AC cap is covered by contaminated sediment, although the remediation efficiencies reduce rapidly with increasing top layer thicknesses. Additionally, adverse effects, such as reduced growth and survival of larvae and a delayed larval development, remain a problem until thicker sediment layers have formed atop the AC cap. In combination with the data from the field trial, these experiments can give valuable information necessary as a basis for the improvement of AC based remediation methods.

WP023 Assimilation Efficiency of Sediment-Bound PCBs Ingested by Fish Is Reduced after Activated Carbon Amendment

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Uptake of polychlorinated biphenyls (PCBs) by fish is controlled by bioavailability of the ingested PCBs in the gut as well as the freely dissolved concentration in the water. Prediction of bioaccumulation in fish relies on models which are often oversimplified. It is also unclear how mitigation strategies affect these models in terms of consumption of PCB contaminated sediment particles. To test the bioavailability of PCBs from treated and untreated sediments, dietary assimilation efficiencies (AEs) were measured for 16 polychlorinated biphenyl (PCB) congeners in mummichogs (Fundulus heteroclitius) that were fed four experimental diets. Diets consisted of PCB-spiked earthworms, untreated sediment mixed with earthworms, activated carbon (AC)-treated sediment mixed with earthworms, and AC mixed with earthworms. AEs were determined by calculating the ratio of PCB mass in the fish tissue to the PCB mass in the food. Fish that were fed the PCB-spiked untreated sediment and those fed AC particles exhibited the highest and lowest AEs over a wide K_{OW} range, respectively. AEs of sediment-bound PCBs were significantly reduced (31 to 93% reduction for different congeners) upon amendment with AC. AEs of PCBs associated with earthworm diet were similar to the values reported in the literature. The present study indicates that assimilation of PCBs can be reduced by enhanced affinity of PCBs to black carbon. The empirical AE-K_d correlations derived in the present study can help improve predictions of sediment contribution to PCB uptake by fish prior to and after addition of carbonaceous sorbents to sediments.

WP024 Effectiveness of activated carbon in reducing the bioavailability of organochlorine contaminants in high organic carbon sediments

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The north shore muck farms of Lake Apopka, FL are contaminated with legacy organochlorine pesticides (e.g., p,p'-DDE, dieldrin, toxaphene, and chlordanes). Sediments at these muck farms have very high total organic carbon content (39%, w/w). The current remediation strategy is to mix the top 15-20 cm of contaminated sediments with the lower 1.2 m of muck soils. This strategy appears to reduce the effective concentrations, but contaminants remain present in the sediments and bioavailable for bioaccumulation into biota. In this study, we assessed the application of activated carbon (AC) as an alternative treatment for the north shore muck farms around Lake Apopka. Sediments from the least contaminated muck farms were spiked with a mixture of 5 chemicals (p,p'-DDE, dieldrin, triclosan, triclocarban, and fipronil) at a nominal concentration of 2 mg/kg chemical, incubated for 7 days to reach a steady state level, and washed unbound chemicals. The spiked sediments were amended with either granular activated carbon (GAC; Fritz) or powdered activated carbon (PAC) pellets (SediMite™) at a dose of 5% of sediment dry weight, mixed with clean water, and incubated for up to 30 days prior to use in exposure experiments. A 7-day sediment bioaccumulation study was conducted using Lumbriculus variegatus (blackworm). The sediments used in the spiking experiments also still contained additional measurable concentrations of weathered pesticides (i.e., p,p'-DDT, p,p'-DDD, and p,p'-DDE). Worm body burdens did not decrease in bioaccumulation experiments with GAC amended sediment and were similar to the concentrations in worms in un-amended sediments after 7 days of exposure. In the experiments using PAC amended sediments, the body burdens in worms of p,p'-DDT, p,p'-DDD, p,p'-DDE, and dieldrin decreased by < 30%, and those of triclosan, triclocarban, and fipronil decreased by >50%, which corresponded to their degree of hydrophobicity (K_{OW} value). These data suggest that the PAC pellets tested here might be an excellent material for in situ remediation of contaminated sediments containing very high background levels of total organic carbon.

WP025 PCB Tissue Concentrations and Benthic Community Impacts at a Carbon Amendment Pilot Study in the Intertidal and Subtidal Zone of San Francisco Bay

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Historical site activities at the Hunters Point Naval Shipyard (HPNS) in South San Francisco Bay resulted in the release of chemicals, including PCBs, to offshore sediments. To inform remedy selection at this urban site, activated carbon (AC) amendments alternatives were evaluated in a pilot treatability study. Two 0.4 acre plots extending from the intertidal to the subtidal zone were treated with either AquaGate + PAC™ or SediMite™ were assessed for their potential to reduce ecological risks associated with PCB-contaminated sediment. Previous treatability studies indicated that AC may be effective at reducing the bioavailability of PCBs to the bent-nose clams (Macoma nasuta) in shallow intertidal sediments when aided by mechanical mixing. This study assessed the effectiveness of AC placements without mechanical mixing in deeper water that is more representative of conditions where full-scale remediation is expected. Tissue bioaccumulation, benthic invertebrate community composition, and chemical analyses were measured as indicators of remedy effectiveness. Comparisons were made between baseline, reference, and post-amendment conditions (6 months and 12 months post-placement). PCB tissue concentrations in Macoma sp. were measured in situ and ex situ (bench-top) after 28-day exposures. Developing field exposure chambers that allowed sediments to infiltrate the chambers and expose clams upon deployment and then retrieve the sediment and exposed organisms for chemical analyses was a challenge. Modifying a chamber design used in previous studies by Luthy et al. (2009) proved successful. Test organisms were another challenge. Tissue bioaccumulation was planned to be conducted with M. nasuta but instead, initial measurements were made with M. secta (white sand clam) collected at a nearby reference location where M. nasuta had been previously found. The species have a similar appearance and life histories but M. secta had low survival in the field (< 20%), lab exposures (< 60%), and lab controls (10%). Additional field pilot testing led to the use M. nasuta from a supplier for post-amendment monitoring. PCB tissue concentrations were reduced by approximately 60% in both pilot amendment areas after 6 months and survival in the field and lab exposures were greater than 90 percent. Benthic invertebrate communities in test plots were not significantly different from baseline conditions or among treatments 6 months after AC deployment.

Developments and Barriers in the Adoption of Amendments for Soil and Sediment Remediation

WP026 Bring contaminated sediment back to life: application of zeolite-amended sediment in soil

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Lake sediment is a crucial composition in lake ecosystem for its role as the storage bank for organic and inorganic nutrients, and for toxics and hazards (i.e. heavy metals) simultaneously. A conventional way of utilizing the lake sediment is to apply it on land to increase soil fertility. However, if the sediment is highly contaminated or not properly managed, heavy metals within the sediment will lead to soil contamination and subsequently cause harmful effects on human beings and organisms. To make such sediment recycled and reusable, a modified zeolite amendment was added to sediment in order to minimize the mobility/bioavailability of heavy metals. The natural zeolite was first modified by chitosan to increase metal adsorption/exchange capacity. The amended sediment was then subjected to BCR sequential extraction scheme. By thorough mixing, the amended sediment was incorporated into soil and impacts of the sediment on soil property were evaluated by water soluble heavy metals (i.e. Cd, Cu, Pb, and Zn), microbial biomass carbon and enzyme activity. The modified sediment had a decreased exchangeable fraction of Cd, Cu, Pb, and Zn, indicating a reduced direct toxicity and effective heavy metal stabilization upon zeolite addition. The soil receiving the stabilized sediment had a reduced water soluble Cd, Cu, Pb, and Zn. In addition, increased total soil microbial biomass carbon and carbon/nitrogen ratio were found in the soil of such treatment. Soil dehydrogenase activity, as a microbial endpoint, was also improved in the soil treated with the modified sediment. Results show the addition of chitosanmodified zeolite as an amendment for contaminated lake sediment was an effective remediation solution. Incorporation of the stabilized sediment into the soil improved soil biological property, and facilitated the recycling and reuse of the sediment at no cost of bringing potential risks associated with the heavy metals.

WP027 Characterizing the partitioning of mercury and methylmercury to activated carbon in the presence of dissolved organic matter

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Mercury in aquatic sediments can pose a significant human health risk when it is converted by microorganisms to the more toxic and bioaccumulative methylmercury (MeHg). MeHg is introduced to aquatic food webs near the sediment-water interface, and can biomagnify to dangerous levels at higher trophic levels. Common remediation strategies for mercury-contaminated sites, including capping and dredging of sediments, can be costly and disruptive and may increase risk by resuspending contaminants or altering redox conditions in a way that favors methylation. Amendment of sediments with activated carbon (AC), an established strategy to decrease the bioavailability of hydrophobic organic contaminants, has shown promise for mercury and methylmercury remediation at the lab scale. AC has the potential to increase solid-phase partitioning and reduce the bioavailability both of inorganic Hg to methylators, and of MeHg to benthic organisms. Quantitative predictions of this partitioning are needed for dose calculations aimed at achieving a target pore water concentration. To date, such AC-water partitioning constants have only been published for the chloride complexes of Hg and MeHg. However, in many estuarine waters, dissolved organic matter (DOM) is much more likely than chloride to control Hg and MeHg speciation, with potentially critical implications for AC remedy effectiveness. We measured the AC-water partitioning of Hg and MeHg complexed to Suwannee River Humic Acid, a well-characterized DOM isolate. Measured logarithmic partitioning constants (L kg^{-1}) were between 3.8 and 4.4, more than two orders of magnitude lower than those published for the chloride complexes. Partitioning was less than 1% similar to that of the chloride complexes, and more than 99% similar to that of DOM alone, in agreement with equilibrum speciation calculations. The results indicate that DOM, a strong and bulky ligand for mercury, can control (and greatly reduce) the overall partitioning of mercury species to AC. At present, we have only a limited understanding of the bioavailability of various Hg and MeHg species and their potential to be attenuated by AC. Nonetheless, in light of this finding, we argue that the effectiveness of an AC amendment will depend heavily on sediment pore water chemistry, and that mercury speciation calculations can be used in combination with partitioning data to improve remedy design and facilitate model predictions of site treatability.

WP028 Decreasing bioavailability of organochlorine pesticides in historical orchard soils

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Repeated applications of organochlorine (OC) pesticides to historical orchard soils pose a continued risk to local ecosystems and fauna. Quantifying this risk, however, has typically focused on total concentration of the contaminant as opposed to its bioavailability. Assessing risk based on bioavailability as opposed to concentration will allow implementation of more economic and practical remediation strategies. For example, various soil amendments have been considered for soils contaminated with the persistent OC pesticides, DDT and dieldrin. In our previous work, locally-sourced composts were shown to reduce the bioavailability of these contaminants to earthworms in contaminated soils from a local historical orchard which is now a CERCLA site. Thus, we expanded this work to examine the changes in the bioavailability of these OC pesticides under field conditions. A small scale plot study was conducted to examine two promising composts under environmental conditions, as well as the long-term effectiveness of the amendments on the bioavailability of these legacy contaminants. Samples of soil and earthworms were collected periodically over three years, and concentrations in both matrices were determined, producing bioavailability data. All earthworms were naturally present in the historically-contaminated site, allowing for real-world bioavailability to be examined. Results showed that although substantial variability due to sampling season and field management were observed, aged-compost was more effective in decreasing bioavailability. These results have lead to the implementation of a 1.5-ha field study. Because this is a long term project, sampling of the field plots will continue in order to meet CERCLA requirements. Finally, the soil sample bank acquired from the plot and field studies will allow for examination of new and developing non-biological methods to determine bioavailability.

Making Your Research Relevant to Regulatory Science and Supportive of Decision-Making

WP029 Activities of the Federal Interagency Workgroup on Pharmaceuticals in Water

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In 2012, four federal agencies signed a Memorandum of Understanding (MOU) establishing a formal mechanism to improve and sustain federal coordination and collaboration on issues related to pharmaceuticals in water. The MOU is in response to the Government Accountability Office recommendation in its August 2011 report "Action Needed to Sustain Agencies' Collaboration on Pharmaceuticals in Drinking Water." The signatories are the US Environmental Protection Agency (EPA), US Department of Agriculture/Agricultural Research Service (USDA), US Department of Health and Human Services/Food and Drug Administration (FDA), and US Department of Interior/Geological Survey (USGS). As a result of this agreement, an interagency workgroup (EPA, USGS, FDA, USDA, Army Public Health Center (Provisional), National Institute of Environmental Health Sciences, National Oceanic and Atmospheric Administration, and Center for Disease Control and Prevention) was formed to address issues related to the occurrence of pharmaceuticals in water. This Workgroup provides a forum for the exchange of information on pharmaceuticals in the environment, supports coordination of joint studies on pharmaceuticals in the environment, and facilitates interagency consultation on implications of research and analyses derived from shared information. The Workgroup is currently developing a product that will summarize ongoing federal efforts in this area and describe the process for monitoring, evaluating, and reporting to the public the results of these efforts. The Workgroup has created a database describing known federal research activities related to pharmaceuticals in water. Potential data gaps were identified and grouped based on the Boxall et al. "Big Questions" paper (2012). Examples of current interagency collaboration to address such data gaps include: developing human health benchmarks for pharmaceuticals, which may help identify pharmaceuticals of greatest risk to humans via the environment; identifying classes of drugs that need to undergo an environmental assessment under the National Environmental Policy Act regulations; studying the effects of chemical mixtures and monitoring pharmaceuticals and other chemicals of emerging concern in water; and developing USDA's five-year project plan. Disclaimer: The views expressed in this poster are those of the authors and do not necessarily represent the views or policies of the U.S. government.

WP030 Development of a practical risk assessment tool for assessing the environmental safety of consumer product ingredients in China (Chera 2.0)

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An environmental risk assessment (ERA) framework and model for consumer product ingredients has recently been developed for China (Chera 2.0). The framework involves an exposure methodology using conditions specific to China including physical setting, infrastructure, and consumer habits and practices. China's current regulatory screening and prioritization schemes are also incorporated as part of a tiered risk assessment approach. The ERA methodology starts with Tier Zero, which utilizes the existing Chinese regulatory qualitative method, whereas Tiers One and Two use deterministic and probabilistic methods respectively, that account for per capita residential water use, wastewater treatment capability, and receiving water dilution factors. Due to major differences in wastewater treatment infrastructure and water usage between urban versus rural regions in China, two scenarios are included for assessing environmental exposure: (1) Urban with wastewater treatment, and (2) Rural without wastewater treatment (i.e., direct-discharge of wastewater). The newly upgraded modeling framework was developed with a Graphical User Interface (GUI) platform that incorporates probabilistic parameters and functionalities. Several ERA case studies will be presented for a variety of consumer product ingredients (e.g., fragrance materials and surfactants) that are widely used in China. These case studies will be used to demonstrate the conservative nature of the Tier 0 assessment and greater environmental realism associated with higher tier methodologies. Future considerations will include enhancing the model's capability with additional GUI features and the inclusion of monitoring network data. The intent of this risk assessment tool is to increase the efficiency for decision-making, which is critical for the management of chemicals in China.

WP031 Integrating weight of evidence into toxicity benchmark development

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Developing toxicology benchmarks for applications in risk assessment is rarely straightforward. Often, interpreting an abundance of toxicology data can be more daunting than dealing with a limited data set. Differences in study design, animal models, methods of administration, variability in response and statistical error all contribute to variations in interpretation. Current methods often assume equivalent quality between studies and available data and use the most sensitive outcome to be protective of others. This is logical for a screening approach; however, when more predictive benchmarks are needed, other methods must be employed. Here we present methods to better assess variability in results and study quality through integration of a semi-quantitative analysis of toxicology data sets. This approach allows for developers to assess the relative value of negative data in an objective manner and begin to assess the influences of false negative and false positive relationships.

WP032 Life Cycle Management on Energy, Water and Material Resources

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Increasing scarcity of material and fossil resources, environmental impacts from sourcing through production and uses, the global competition on limited and high valued materials for the high technology sectors and the increasing costs are urgent drivers for improving sustainable production, reducing emissions and pollution and supporting environmental protection startegies. The working conditions of employees are challenged through sourcing processing on limited raw materials, increasing competitive production in well-developed countries but also the need to stimulate economic and social development in transition and developing countries. Life Cycle Assessment (LCA) and Life Cycle Costing (LCC) will be used for setting Sustainability criteria of products. Special focus will be addressed to Energy, Material and Water Resources during the life stages sourcing, production and uses. LCC assessment will determine the performance criteria of achievable BAT and BEP of products. Re-use and recycling is of growing importance for limited and and high value materials such as noble earths. LCA-based methodology is being used as management tool for decision-making. This Life Cycle Management (LCM) serves as an instrument to make LCA and LC-Tools operational. LCA studies were developed more than 80 priority product groups. The selected products are characterized by high market volume, environmental relevance and potentials for improvement. Management Systems on Resource Efficiency such as Energy, Materials and Water Resource Management procedurally follow the same systematic and operational approach of LCA and LCM. Typically, data measurement, compilation, analysis and assessment of data as well as improvement recommendations are main components of these Management Systems. But also training and qualification of personnel, organization, communication and a continual improvement process are important elements to reach a sustainable processes,improved technologies and products. Holistic approaches provide transparency and credibility for assessments. There is a number of cases where Life Cycle Management (LCM) provided knowledge-based and scientifically defendable decision options. The political decision OSPAR on the Decommissioning of Offshore Oil/Gas Installations in 1995/98 were based on extensive LCA studies and the application of LCM tools.

WP033 Policy Shaping Flame Retardant Use and Regulation: the Role of States and Future under TSCA Reform

E. Schreder, Washington Toxics Coalition

At the federal level in the United States, the U.S. Environmental Protection Agency (USEPA) has had very limited authority to restrict chemicals under the Toxic Substances Control Act (TSCA) of 1976. Therefore, since 2003 when California passed the first state-level restrictions on the use of flame retardants, states have taken the lead in the United States in identifying and acting to restrict flame retardants that pose a hazard to human and environmental health. State legislatures have passed laws to restrict primarily focused on two classes of flame retardants, PBDEs and chlorinated organophosphates. As these laws have come into effect, a pattern has emerged in which one to several states restricts a flame retardant, then manufacturers announce voluntary withdrawal of that chemical from the market. In some cases, this has involved agreements made with the USEPA and federal rules restricting future production. The June 2016 passage of TSCA reform by the U.S. Congress is likely to change this dynamic somewhat: the USEPA will now be required to adhere to a schedule to assess chemicals, but the new law places constraints on the ability of states to restrict chemicals. USEPA has started the process of scoping risk assessments for four types of flame retardants: chlorinated phosphate esters, hexabromocyclododecanes, tetrabromobisphenol A, and brominated phthalates. The history of state policy action on flame retardants was reviewed and an analysis developed of the expected effect of new authority and actions at the federal level.

WP034 Strategy and development of direct-discharge assessment methodology for consumer product chemicals

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The direct disposal of untreated wastewater (or very limited treatment) into the environment is a common practice in many regions around the globe, particularly in rural areas of Asia, Central and Eastern Europe, and Latin America. Elevated levels of organic wastes from untreated wastewater cause an increase in oxygen demand due to the concomitant increase in biological decomposition. The zone of impact can be characterized by relatively high total suspended solids (TSS), and biochemical oxygen demand/chemical oxygen demand (BOD/COD), resulting in low dissolved oxygen and high ammonia concentrations, which itself can be inhibitory to aquatic organisms. Thus, risk assessments for consumer product ingredients under these exposure scenarios should take into account such conditions with reduced DO and increased ammonia due to conventional pollutants. In our risk assessment framework, we: (1) used a mechanistic modeling scheme for estimating water quality to delineate the zone of impact and subsequent risk level for chemicals of interest; and (2) compared our approach to more traditional and established methods. In particular we evaluate whether our approach can be used under broad or specific conditions. Furthermore, chemical and fate properties (e.g., biodegradability and sorptivity) and environmental factors (cold, temperate or tropic regions and effluent/receiving water dilution) where consumer products are used and disposed of will be analyzed to assess their interactions for affecting the size of the impact zone and risk assessment outcomes. A series of case studies using commonly used consumer product ingredients (e.g., fragrances, surfactants) illustrate this proposed assessment strategy with representative scenarios for China, South America, and Eastern Europe. Comparison of these case studies will provide an opportunity to recommend guidance in appropriately assessing the impact of down-the-drain consumer product ingredients in direct discharge scenarios globally.

Microplastics in the Aquatic Environment: Fate and Effects

WP035 State of the Science Overview: Effects of Plastics Pollution on Aquatic Life and Aquatic-Dependent Wildlife

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Plastic production is increasing globally, with the volume of plastics entering marine and freshwater ecosystems increasing by several orders of magnitude since mass production began in the 1940s. The adverse physical impacts to organisms from plastic debris in aquatic environments, including ingestion and entanglement, have been well documented, and the ingestion of plastics may establish an exposure pathway for chemical contaminants sorbed from the water column or incorporated into the plastics during manufacture. The U.S. Environmental Protection Agency (EPA) Office of Water developed a state-of-the-science review of the chemical effects associated with plastic pollution and potential impacts on aquatic life and aquatic-dependent wildlife. Based on a review of the literature, available research from field and experimental studies suggests invertebrates, fish, and birds can accumulate chemicals associated with ingested plastics. Toxicological studies with invertebrates and fish have shown sublethal effects from chemicals associated with plastics as well as the plastic itself. However, bioaccumulation modeling under environmentally realistic exposure scenarios provides indirect evidence that plastics serve as a relatively minor contaminant exposure pathway compared to other natural pathways. Based on the current state-of-the-science, there are significant opportunities for research to further our understanding of the potential toxicological impacts of plastic ingestion throughout the food web. In addition, EPA's Trash Free Waters program may be a catalyst for proactive plastics reduction by helping states and localities reduce the volume of plastics, trash, and debris that enters both freshwater and coastal ecosystems.

WP036 Microplastics as Vectors of Polycyclic Aromatic Hydrocarbons (PAHs) via Chemical Adsorption and Desorption

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With an approximate degradation time of 450 years, plastic is one of the most durable and persistent environmental contaminants in the modern world. Production since the 1950s has increased prodigiously. In the marine environment, photooxidative degradation and the abrasive action of waves progressively degrade larger pieces of plastic into tiny polymer particles less than 5 millimetres in diameter, termed microplastics. As a consequence of their small size, microplastics are inadvertently ingested by ocean dwelling biota, particularly susceptible filter feeders such as Mytilus edulis and many species of fish. This can result in detrimental effects such as inhibition of gastrointestinal function and feeding impairment. However, it has emerged fairly recently that microplastic polymers collected from the marine environment have been found to have hydrophobic organic chemicals (HOCs), such as polycyclic aromatic hydrocarbons (PAHs), adsorbed onto their surface. Since marine organisms ingest microplastics, it can be proposed that microplastics have the potential to act as a transporter for the conveyance of persistent organic pollutants (POPs), such as PAHs, into marine biota. PAHs are formed from incomplete or inefficient combustion of organic material, diagenesis and biosynthesis. While there is a consistent background level from forest fires and volcanic activity, a significant fraction of PAHs present in the environment is due to anthropogenic activities, such as internal combustion engines. Consequently, PAHs reach the marine environment via sewage and industrial discharges, oil spillages and deposition from the atmosphere. One particular characteristic of PAHs is their susceptibility to ultra-violet light. However, pericondensed PAH structures are more centrally condensed allowing them to withstand higher ultraviolet fluxes. This results in a decrease in susceptibility to photodecomposition by ultraviolet light and thus, resistance to degradation in the marine environment. Consequently, microplastics can then adsorb these contaminants. Since many of the susceptible organisms that ingest microplastics are located at the bottom of the food chain, chemical adsorption provides a potential mechanism for the bioaccumulation of contaminants in marine organisms and the potential trophic transfer of these contaminants up the food web to larger organisms, and possibly humans. Consequently, it is necessary to investigate this potential mechanism.

WP037 Microplastics and nanoplastics in South African Aquatic Systems: Occurrence and Distribution

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Microplastics and nanoplastics water pollution research in South Africa should be in the forefront of water research, as they represent a basket of aquatic system contaminants which has been classified as emergent and has proved to have entered into the food chain. The utilisation of the South African coastal areas to boost the much required economic development, through trade ports; tourism; fish; and seafood industries makes it imperative to investigate the abundance, occurrence, distribution and accumulation of microplastics and nanoplastics pollution in rivers, dams, lakes, ocean, sea, estuaries and beach environments. Almost all publicly provided potable water in South Africa originates from rivers, dams, and groundwater. Unfortunately the associated environmental risks including comprehensive data on microplastics and nanoplastics' occurrence and distribution including biological effects are currently lacking in South Africa just as it may be the case for other countries. The objective of this paper is to presenton the status of research and literature generated in South Africa from a quantitative and qualitative data's perspective in demonstrating the abundance, occurrence and distribution of microplastics in the South African oceans, beaches, estuaries and freshwater systems. The other objective is to improve knowledge on the occurrence, distribution and possible accumulation in South African freshwater system. The South African historical political situation and the current migration trends towards urban areas has created unintended problems that manifest themselves though the mismanagement of a large quantity of urban litter that includes microplastics and nanoplastics. Plastics, microplastics and nanoplastics research in South Africa started in the late 80's, with a lot of work focused on the coastal areas, but work on freshwater systems is lacking and this fragmentation of knowledge reduces the chances of achieving a transdisciplinary directed risk assessment for freshwater systems. One major challenge in microplastic research is the lack of general definitions and convergence in methodology. The majority of South African researchers have utilised similar methodology for sample processing, however, the methodology utilised for sample collection was different. This might be of concern because, differing methodologies may sometimes lead to data interpretation divergence. A compromise interpretation model has been proposed in this review.

WP038 Ecological investigations of microplastics and microparticles in environmental matrices-environmental science is now in the second (not first) wave

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Investigations of the environmental fate and effects of microplastics and microparticles is now in vogue. Small, insoluble polymeric particles are implicated by scientists in a wide variety of studies that are used to suggest potential for wide-spread impacts in freshwater and marine pelagic and sediment environments. In the past 7 years, the annual rate of publications regarding environmental fate, exposure, bioaccumulation and ecotoxicity of microplastics has grown nearly 50-100 fold. This literature has ignored deep and important literature on the use of microplastic (microsphere) particles to understand the feeding biology, behavior, and ecology of zooplankton, epibenthic invertebrates and early life stages of fish. Much of this rich literature is found in limnological and marine biology and ecology studies of invertebrates in the 1970's to early 1990's. The types of research that was performed in this arena includes studies to develop an understanding of size selectivity of filter-feeding zooplankton, clams, mussels, early development stages of echinoids; evaluation of particle sorting efficiency and ingestion rates of zooplankton; determination of filtering ability of organisms as a surrogate for classic chronic toxicity endpoints for zooplankton; use of functionalized particles tagged with flavors to address taste discrimination by zooplankton; investigation of microplastics as potential mosquito larvicides when applied as a physical barrier on pond surfaces; understanding the movement of microparticles in sediment matrices following bioturbation. In this paper, we review this literature and what it can do to inform scientists on the currently emerging information on microparticle/microplastic ecotoxicity as a comparison.

WP039 Zooplankton exposure to microplastic at estuarine tidal fronts in Charleston Harbor, SC

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The Charleston Harbor in Charleston, SC receives input from three main river systems and the Atlantic Ocean, generating tidally-driven frontal aggregation zones for semi-buoyant particulates. Microplastics are found in Charleston Harbor and have entered the estuarine trophic system as evidenced by fragments and fibers found in the guts of shrimp, the feces of local birds, and the gills of Eastern oysters, Crassostrea virginica. The present study examined whether aggregation zones at flood tidal fronts in the Ashley and Cooper River present a pathway of increased exposure for microplastics to enter the estuarine trophic system via ingestion by zooplankton. Fluorescence and bright-field microscopy were used to enumerate microplastics in filtered surface water samples and melting point analysis confirmed the identification. The increased relative abundance of microplastics in flood tidal fronts compared to the surrounding water suggests these systems concentrate microplastics similarly to phytoplankton and thus may be an important conduit for microplastic to enter the food web. Additionally, each front and associated river system may be characterized by different relative abundances of microplastic fibers verses fragments. Future analysis will examine zooplankton populations collected at tidal fronts to provide a better understanding of the movement of microplastics into the lower trophic feeding guilds in a dynamic estuarine environment.

WP040 Microplastics in a surface water environment receiving treated wastewater effluent

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The large global production and use of plastics has led to the presence and accumulation of plastic particles in aquatic environments. The presence and impact of plastic debris, particularly in the oceans, has been highly publicized; it has been proposed that plastic debris can adversely affect marine organisms physically and chemically. Plastic polymers show little environmental degradation, although over time the size of plastic particles decreases. These small plastic fragments (microplastics or solid particles less than 5 mm in size) accumulate in surface water environments. In addition, microplastics or "microbeads" used in personal care products and cosmetics may ultimately find their way into aquatic environments. Ingestion of microplastics by filter feeding organisms has been observed, but the significance of this exposure is unclear. A recent paper (Lönnstedt and Eklöv, 2016) indicated impacts of microplastics on larval fish behavior and development. Microplastics are also present in freshwater environments where they may have similar impacts. We measured the presence of microplastics (53 – 106 µm) spatially and temporally in a series of connected urban lakes fed by treated wastewater effluent. The lakes also serve as urban drainage during storm events. We used wet sieving of water similar to methods described by NOAA. A sample of treated wastewater effluent feeding Lake 1 (3.75 L) contained 10 mg of microplastics. A near-surface sample of similar volume from the dam at Lake 1 contained 7 mg of microplastics. A sample of water flowing into the 7th lake in the series contained 15 mg of microplastics. These results suggest that urban runoff contributes microplastics to the system in addition to the treated wastewater effluent. Recent legislation has been proposed that would ban microbeads in personal care products and cosmetics by July, 2017. Our preliminary data suggest that additional monitoring would help to better understand such regulation, the potential impacts of historic use, and the input to aquatic environments of other sources of microplastics beyond personal care products.

WP041 Identifying Point Sources of Microplastic Debris in Charleston Harbor, SC

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Plastic marine debris has been identified as a high-priority research area due to the worldwide prevalence of plastic debris in the marine environment. Of particular interest is the occurrence of small plastic particles measuring less than 5 mm in diameter. These particles, termed microplastics, are considered an emerging contaminant of concern. Accordingly, there is a growing effort to determine the origin and fate of microplastics in aquatic ecosystems. In order to identify point sources of microplastic pollution entering Charleston Harbor, South Carolina, industrial effluent discharged into the Cooper River, a tidal river emptying into Charleston Harbor, was analyzed for the presence of microplastics (63-500 µm). In addition, the distribution and abundance of microplastics in the Cooper River was determined. These data help to elucidate the role of riverine input as a source of microplastic in Charleston Harbor, and can help us to understand the fate of microplastics in an estuarine ecosystem.

WP042 Contribution of Plastic Debris to Microplastic Abundance in Charleston Harbor

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To mitigate the problems associated with microplastic in the environment, it is important to understand their sources and the pathways by which they gain entry into various habitats. Previous studies in our laboratory have demonstrated that the major type of microplastic particle found in Charleston Harbor are fragments consistent with the degradation of plastic debris. To understand the mechanisms involved in the formation of these particles and to identify possible sources, three studies were conducted. First, plastic strips composed of high density polyethylene, polypropylene, and polystyrene were deployed in a salt marsh, and monitored for 32 weeks. A laboratory mechanical fragmentation test demonstrated that the production of microplastic particles from these strips was evident following 8 weeks for all three polymers. SEM images suggested that the production of these microplastic particles resulted from the delamination of the surface layer. To validate these initial findings, a second study was conducted examining microplastic production from eight discarded plastic debris items found in situ in a salt marsh. In each case, there was significantly more microplastic particles produced in the laboratory mechanical fragmentation test by the field-collected, discarded plastic debris relative to the non-discarded control material. To identify possible sources of black microplastic fragments, the major type of fragment found within the harbor, a third study was conducted examining microplastic production from field-deployed strips of common black plastic items (oyster reef restoration bags, silt fencing, monofilament fishing line, fishing net, and grocery bags). At 16 weeks, monofilament fishing line and the fishing net produced the greatest number of microplastic particles, and silt fencing produced the least. These results suggest that nylon fishing gear may be a major contributor to the microplastic litter in Charleston Harbor.

WP043 An estimation of plastic debris and persistent and bioaccumulating toxic riders in the Tijuana River Estuary

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Accumulation of plastic debris in aquatic environments is a growing concern. Recent research has demonstrated that small plastic beads, fragments and particles, or "microplastics," can pose chemical exposure hazards as well as physical hazards to aquatic animals. Microplastics have been found to attract, harbor and hyperaccumulate Persistent and Bioaccumulating Toxins, thereby acting as another exposure media to birds, fish and macroinvertebrates, including species we consider seafood. We separated plastic debris pieces from sediments at the Goat Canyon Retention Basin (GRCB) at the Tijuana River Estuarine Natural Resource Reserve. We estimated plastic quantities in sediment samples and analyzed sediments and plastic fragments for PBTs using a Gas Chromatography/High Resolution Mass Spectroscopy (GC/HRMS). We compared chemical concentrations between sediments and co-located plastic fragments. Concentrations of PBTs on plastics far exceeded concentrations in sediments from the same sample location. PCB results on plastics also exceeded sediment contamination threshold criteria. A "back-of-the-envelope" calculation yielded an estimate of 21.3 to 41.3 metric tons of plastic in the GCRB alone with a total PCB load of 4 to 7 kg. These findings suggest that typical PBT risk assessments performed on sediments may be confounded by the presence of contamination on entrained microplastic media. Further exploration of this phenomenon could have significant impacts on contaminated sediment risk assessment methodology and decisions regarding cleanup and management decisions such as invasive sediment exaction activities.

WP044 Assessment of environmental fate of solid polymers in wastewater treatment plant

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Over the last several years there have been a range of scientific and non-technical articles reporting the presence of microplastics in rivers, lakes, and oceans. Although the direct source for input of these particles into waterways is not clear, it has been suggested that the ability of these micron and sub-micron particles to pass through wastewater treatment may contribute towards microplastic's input into the environment. However, most well-functioning municipal wastewater treatment plants (WWTP) remove solids from the wastes stream by sedimentation during both primary and secondary treatment. Sedimentation simply entails the physical settling of matter, due to its density, buoyancy, and the force of gravity. Chemical coagulants and flocculants are often used to improve the efficiency of this process by facilitating the aggregation of particles. To address the environmental fate of two solid polymers, SunSpheres™ and Opulyn™ 301, we initiated a laboratory study to evaluate whether these materials can be removed from wastewater during sludge settling. This laboratory study was not meant to directly simulate the operating conditions in WWTPs, but to provide preliminary first tier screening level estimates on the removal potential of SunSpheres™ and Opulyn™ 301 during sludge settling in a typical municipal WWTP. Wastewater containing activated sludge was collected from the oxidation ditch bioreactor at a Municipal Wastewater Treatment Plant one day prior to the initiation of the test, and was continuously aerated until use. Aliquots of SunSpheres™ PGL Polymer and Opulyn™ 301 were added at a range of concentrations into the wastewater. The dosed wastewater was mixed at 100 rpm for ~ 2 hr, and subsequently settled for 3 – 4 hr. The mixing and settling time was chosen to reflect the actual retention/detention time of an aeration tank and a secondary clarifier in a typical municipal WWTP. After settling, the upper aqueous phase was analyzed by pyrolysis coupled with GC/FID for SunSpheres™ PGL Polymer and Opulyn™ 301. Removal of SunSpheres™ and Opulyn™ 301 from wastewater was ~ 99%. This laboratory study was not meant to directly simulate the operating conditions in WWTPs, but the results clearly demonstrated that SunSpheres™ and Opulyn™ 301 have great potential to be removed during sludge settling in a typical municipal WWTP.

WP046 Differential bioavailability of PCBs associated with environmental particles: microplastic in comparison to wood, coal and biochar

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Microplastic particles are increasingly being discovered in diverse habitats and a host of species are found to ingest them. Since plastics are known to sorb hydrophobic organic contaminants (HOCs) there is a question of what risk of chemical exposure is posed to aquatic biota from microplastic-associated contaminants. We investigate bioavailability of polychlorinated biphenyls (PCBs) from polypropylene microplastic by measuring solid-water distribution coefficients, gut fluid solubilization, and bioaccumulation using sediment invertebrate worms as a test system. The results are compared to several natural and anthrogenic particles, including wood, coal, and biochar, placing microplastic-associated PCBs in a differential bioavailability framework. PCB distribution coefficients for polypropelene were higher than natural organic materials like wood, but in the range of lipids and sediment organic carbon, and much smaller than for black carbons like coal and biochars. Gut fluid solubilization potential increased in the order: coal < polypropylene < biochar < wood. Interestingly, lower gut fluid solubilization for polypropylene than biochar infers that PCBs at the biochar surfaces were accessible to gut fluid micelles while bioaccessibility is limited by diffusion from the interior of the microplastic. Biouptake in worms was lower by 76% when PCBs were associated with polypropelene compared to sediment. The presence of microplastics in sediments has an overall impact of reducing bioavailability and the transfer of HOCs to sediment-ingesting organisms. Since the vast majority of sediment and suspended particles in the environment are natural organic and inorganic materials, pollutant transfer through particle ingestion is going to be dominated by these particles and not microplastics. Therefore, these results support the conclusion that in most cases the transfer of organic pollutants to aquatic organisms from microplastic in the diet is likely a small contribution compared to other natural pathways of exposure.

WP048 Plastic Pollution as a Transport Mechanism for PCB Loadings in the Hawaiian Monk Seal at Tern Island/French Frigate Shoals

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Tern Island/French Frigate Shoals (FFS) is designated critical habitat for the endangered Hawaiian monk seal and is also the largest pupping grounds in the Northwestern Hawaiian Islands (NWHI) for the monk seal. Approximately 95% of the threatened Hawaiian green turtle population is known to nest in the FFS, including Tern Island. FFS is also home to approximately 6% of the world's population of the endangered black footed albatross and is a long-term monitoring site for 18 breeding seabird species – numbering approximately 500,000 birds – making Tern Island the most species-rich rookery in the NWHI. Studies conducted on Tern Island and its immediate vicinity have concluded that there is clear evidence of bioaccumulation through the aquatic food chain of high concentrations of PCBs and other lipophilic contaminants in organisms that occupy high trophic levels. In response to a December 2012 petition from the Center for Biological Diversity, EPA in partnership with FWS conducted an evaluation of releases, or threats of release, of hazardous substances from Tern Island that may adsorb to plastic marine debris in the surrounding surface water. Microplastic particles may provide a mechanism to concentrate and transport hazardous substances to marine species via the food chain. High levels of PCBs in the blood and blubber of the Hawaiian monk seal are a likely stressor contributing to the precipitous decline of the species. Microplastic ingestion via the food chain is a likely pathway for PCBs to enter the bodies of the monk seal and EPA is committed to understanding the role and relative threat posed by marine plastic pollution on the health of this endangered mammal. Initial steps to conduct a remedial investigation and risk assessment have begun, with special focus on the toxicological impacts, including PCB uptake, from contaminated plastic ingestion on the prey which make up the monk seal diet. The information gleaned from determining the risk posed by plastic particle pollution to the US' most endangered marine mammal may be transferrable to the effects and impacts expected on other mammals who eat seafood, such as humans.

WP049 Predicting the Ecological Implications of Leachates from North Pacific Gyre Plastics using In Vitro and In Vivo Models

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Marine plastics are one of the most common and persistent pollutants in ocean waters and beaches worldwide and are estimated to be present between 60-95% in the marine environment. Plastic marine debris may pose a threat to aquatic life as many plastics contain endocrine-disrupting compounds including nonylphenol, bisphenol A and various phthalates, and have been shown to adsorb common persistent organic pollutants. Plastic samples recovered from the North Pacific Gyre along with UV-irradiated virgin plastic and non-irradiated virgin plastic were extracted and concentrated using solid phase extraction (in methanol) and nitrogen evaporation. In vitro assays using luciferase-reporter estrogen receptor-dependent (ER) agonist cells (VM7Luc4E2) revealed significantly higher activity in UV-irradiated virgin plastic leachates than gyre plastics and virgin plastics (EEQ= 159.0, 7.7 and < 0.1 ng/L , respectively). Beta-lactamase-reporter GeneBLAzer® CYP1A1-Bla (aryl hydrocarbon-receptor or AhR) assays revealed greater activity in gyre-recovered plastics than in virgin plastic and UV-irradiated virgin plastic (TEQ= 2, < 0.1 and < 0.1 ng/L, respectively). Oryzias latipes (Japanese medaka) fish were used as an endocrine-disruption in vivo model for these plastic leachates. To better understand the in vivo responses of plastic leachates and adsorbents, larval Japanese medaka (1-5 dph) were exposed to 0.01% methanol plastic extracts for 5 days. Induction of vitellogenin mRNA (VTG; an estrogen-dependent yolk precursor) and cytochrome P450-1A mRNA (CYP1A; an aryl-hydrocarbon-dependent enzyme) were measured using quantitative real-time polymerase chain reaction (q-RT-PCR). Delta-Ct values for CYP1A showed a significant difference between control and ocean plastic-treated fish (p=0.033, dunnett test), with an expression fold change of 10.7. Irradiated virgin plastic-treated fish showed no significant differences for either gene tested.

WP050 Ingestion of experimentally contaminated microplastics causes changes in biomarker responses in three-spine stickleback Gasterosteus aculeatus

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Microplastics (MPs) raise great environmental concerns due to the wide distribution in aquatic environment and its known capacity to absorb and concentrate environmental contaminants. As potential threats of MPs are greatly unknown, research oriented towards understanding of biological fate of MPs in organisms and their effects, is greatly needed. A two-tier experimental study was conducted to study vector effects of MPs following different environmentally relevant exposure scenarios. Unplasticized polyvinyl chloride (uPVC) microscopic pellets were exposed to either model compounds, such as bisphenol A and crude North Sea oil, as well as to environmental mixtures (sewage effluent and industrial harbor's water). Consequently, long-term dietary exposures utilizing pre-exposed particles (incorporated in fish diet) were performed on three-spine sticklebacks (Gasterosteus aculeatus). The dietary toxicity of MPs and associated chemicals on fish was assessed using biomarkers' approach in two subsequent feeding experiments. Findings of the study provided strong evidences of MPs as vectors for environmental pollutants, transferring desorbed chemicals into fish. Results revealed that uPVC MPs may not be toxic alone, but when co-exposed to environmental hazards, mediate hepatotoxicity and act as endocrine disruptors. It was demonstrated that experimentally contaminated MPs cause changes in EROD activity and gene expression in liver, specifically altering gene expression of detoxification enzyme CYP1a, metallothionein (MT), estrogen receptor α (ER-α), vitellogenin (VTG) and androgen receptor β (AR- β). Changes in enzymatic biomarker responses were detected in gills, suggesting gills to be an important site for MPs-related toxicant exposure.

WP051 Effects of acute exposure of microplastics on the physiology of blue mussels

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As sedentary organisms, suspension-feeding bivalves are particularly vulnerable to anthropogenic contaminants that enter coastal environments. To understand the full range of impacts that contaminants such as microplastics have on these animals, sub-lethal effects must be assessed. In this study we exposed the blue mussel, Mytilus edulis, to microspheres composed of polystyrene divinylbenzene (ca. 3-30 µm; 1500 microspheres ml^{-1}, Bangs Labs) for a period of 12 hours. Prior to exposure, mussels were delivered a microalgal diet for 1 day and then divided into two groups: a microplastic-exposed group and an unexposed control group. Mussels in each group were then placed in individual feeding chambers supplied with the microalgal diets and microspheres as appropriate. After exposure, three physiological parameters were measured including diet absorption efficiency (AE; Conover 1966), oxygen consumption, and ammonium excretion (phenolhypochlorite method). Each mussel was then sacrificed and soft tissues isolated and dried to a constant mass. All physiological parameters were then standardized to a 1-g dry tissue mass using appropriate allometric equations. Absorption efficiency of mussels exposed to microplastics (55.6%) was significantly lower than AE of control mussels delivered only the microalgal diet (73.6%). No significant differences were found between treatments for mussel oxygen consumption or ammonium excretion. Results indicate that acute exposure of mussels to polystyrene-divinylbenzene microplastics can have significant impacts on digestive processes, but not on respiration or ammonium excretion rates.

Advances in Exposure Modeling: Bridging the Gap Between Research and Application

WP052 Human exposure to household cleaning products: Application of a two-field model

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Indoor cleaning is a common household activity, and cleaning products contain volatile organic compounds that constitute a potential threat to indoor air quality and occupants' health. This study aims 1) to establish a dynamic modeling framework which accounts for the near-person inhalation and dermal exposure to indoor cleaning products; 2) to determine the evolution of chemical mass and concentration associated with the activity, and to determine the factors affecting chemical fate and exposure in indoor environment; and 3) to determine the short-term human intakes and product intake fractions. We modify a two-zone model to describe the indoor environment where cleaning products are applied, identifying four transfer compartments, i.e. near-person surface, near-person air, far-person surface, and far-person air. Mass transfers between compartments are described by first-order transfer rate constants structured in the K matrix. The exposure matrix, XP matrix, relates the mass in a given compartment to the intake by human. Three exposure pathways are considered: inhalation exposure to near-person air, inhalation exposure to far-person air, and dermal exposure during application. The model is applied to 20 common ingredients in surface cleaning products, with a focus on two representative chemicals, n-hexane (high volatility) and 2-butoxyethanol (low volatility). The application of cleaning product is assumed to last for 1.5 hours (Phase 1) and a time period of 200 hours after application (Phase 2). For both representative chemicals, mass in each compartment continues increasing during Phase 1 and reaches its peak at the end of Phase 1. However, for n-hexane the two air compartments have higher chemical masses after the application, whereas the masses in the two surface compartments are higher for 2-butoxyethanol. Dermal intake during Phase 1 dominates exposure for n-hexane, while inhalation exposure during Phase 2 dominates for 2-butoxyethanol. Among the studied chemicals, inhalation intake dominates the total product intake in most cases and is driven by the chemical's air-water partition coefficient. The adapted dynamic two-zone model describes with parsimony the dynamic of chemical mass and near-field intakes during the application of household surface cleaning products. The results will be compared to exposure estimates for application of other cleaning products to elucidate the relative importance of various transfer pathways in near-field exposures.

WP053 A spatial approach for estimating the national distribution of sewer residence times for wastewaters in the US

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Sewer residence time can have a significant influence on the environmental fate and transport of wastewater constituents, including down-the-drain household consumer product ingredients. In this study, best-available data resources and geoprocessing tools were used to develop a spatial approach for estimating the national distribution of sewer residence times for wastewaters in the U.S. Case studies estimating sewer residence times for two municipalities demonstrated that road networks could be used as a spatial proxy for sewer networks when the latter data is not available. The approach was then extrapolated to a national dataset of >3,400 wastewater treatment plant (WWTP) facilities across the U.S. to estimate the national distribution of sewer residence times, with an estimated national median sewer residence time of 3.3 hours. Sewer residence times for smaller WWTP facilities (< 1 million gallons per day) were comparatively shorter than larger facilities, however the latter comprised a greater proportion of the overall national wastewater volume. The sewer residence time distributions derived in this study can be combined with in-sewer biodegradation data to estimate WWTP influent concentrations of down-the-drain household consumer product ingredients as part of a national-scale probabilistic risk assessment.

WP054 Global-Scale Multimedia Chemical Fate Modeling in High Spatial Resolution: Introducing BETR-Research 3.0

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BETR-Research is a framework to create spatially resolved multimedia fate and transport models for chemical contaminants. The first version of BETR-Research was based on BETR-Global 2.0, which is a well-developed and tested global fate model implemented in Visual Basic for Applications (MacLeod et al., 2011). BETR-Research was developed by re-implementing BETR-Global 2.0 in the Python programming language. The purpose of this re-implementation was to create a more flexible modeling platform using BETR-Global's model structure and taking advantage of efficient numerical packages in Python. In recent years, the BETR-Research model source code has gone through several important modifications. Some of the chemical fate process descriptions have been modified, a new algorithm for tracking chemical mass transfer fluxes throughout the simulations has been added, and a fast differential equation solver library to be used in dynamic model simulations has been integrated to the model code. Also, the databases that describe global atmospheric and oceanic flows, and climate properties have been updated; and now interannual variability can be accounted for. Further, there are options for quantifying the contribution of secondary emissions to atmospheric concentrations. The new version of BETR-Research can run global model simulations in spatial resolutions of 7.5°×7.5° and 3.75°×3.75°, in addition to the 15°×15° base resolution of BETR-Global. Here, we demonstrate BETR-Research 3.0 by simulating the global distribution of two polychlorinated biphenyls (PCB28 and PCB153) in 3.75°×3.75° spatial resolution. This application is a high-resolution upgrade of a previous modeling study using BETR-Global (Lamon et al., 2009). This model application was used to verify the implementation and new process descriptions, and to establish that the high-resolution environmental data set is reliable for use in future BETR-Research global modeling studies. The model results were compared with updated monitoring data sets and the model performance was evaluated. The source code of BETR-Research 3.0 will be publically available through an updated website which will also include the new high-resolution global environmental input data sets.

WP055 Geospatial Modeling of Particulate Matter Pollution from Post-Panamax Ships in Charleston Harbor, SC

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The US Army Corps of Engineers (USACE) has been selected by South Carolina State Ports Authority to dredge the Charleston Harbor (CH) to a depth of 52 feet from the current depth of 45 feet (i.e. Post 45 Project). The completion of the Post 45 Project will make CH the deepest harbor on the East Coast which enhances the economic efficiency of commercial navigation and the local economy. The deepening of the port will allow for the passage and docking of Post-Panamax (PP) container ships which can carry ~1,000 additional cargo containers as compared to Panamax containers ships. The USACE environmental impact statement air quality assessment only calculated the emissions at four terminal locations and stated that total terminal emissions would be less than the status quo due to the use of larger and more efficient container ships. As part of the larger Integrated Risk Assessment and Vulnerability Assessment project, the presented work details the models and analysis used to conduct a geospatial comparative release and exposure assessment of particulate matter (PM) from Panamax and PP container ship traffic in CH. Our analysis extends beyond that of the USACE EIS as it includes the use of geographic information system software to determine potentially impacted populations by overlaying the container ships' funnel plume PM dispersion with population data and other sociodemographic data. The analysis is organized into four sections: pre-dredge PM emissions modeling of Panamax container ships moving through the harbor to the shipyard, pre-dredge PM emissions modeling of Panamax container ships idling at the shipyard, post-dredge PM emissions modeling of Panamax and PP container ships moving through the harbor to the shipyard, and post-dredge PM emissions modeling of Panamax and PP container ships idling at the shipyard. Various atmospheric dispersion models are employed in an effort to find the model or set of models (e.g. Eulerian, Lagrangian, and Gaussian dispersion models) that best represents the characteristics of the container ships' emissions and atmospheric profile of CH. Future work includes modeling the dispersion of other criteria pollutants and pollutants of interests emitted by the container ships. The goal of this research is to understand the potential geospatial air pollution and human health impacts of the Post 45 Project on Charleston Harbor and compare the findings to the results presented in the USACE environmental impact statement.

WP056 Sorption of non-ionic and anionic surfactants to three stationary phases as parameters for QSAR models

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Anionic and non-ionic surfactants are high-production volume chemicals which are found in many consumer products and consequently also in the environment. The octanol-water partition coefficient (K_{ow}) is often used in QSAR studies as a measure of hydrophobicity to predict the environmental fate and exposure of neutral organic compounds. However, K_{ow} is not meaningful for surface-active compounds because sorption to environmental matrices is not only driven by their hydrophobicity, but also by specific interactions with the sorbent (hydrogen-bonding and electrostatic interactions). Furthermore, experimental K_{ow} values of both non-ionic and anionic surfactants are difficult to determine because of their surface active properties and ability to emulsify the octanol-water system. Alternative approaches to quantify and predict the environmental behavior of surfactants are therefore required. Our research is focused on developing and testing new approaches and parameters that can be applied in models to predict sorption and bioaccumulation of different surfactant classes. To this end we studied the interactions between surfactants and different stationary phases with liquid chromatography: C_{18}, mixed-mode hydrophilic interaction (HILIC), and mixed-mode weak anion exchange (WAX). To quantify the contribution of hydrophobic, hydrogen-bonding and electrostatic interactions to the environmental behavior of surfactants, capacity factors were derived from the different stationary phases. The affinity of surfactants for the C_{18} phase increased with carbon chain length for both anionic and non-ionic surfactants. The non-ionic surfactant class of alcohol ethoxylates showed increased affinity with the mixed-mode HILIC phase when increasing the number of ethoxylate units in the molecule. The logarithmic capacity factors determined for alcohol ethoxylates with both C_{18} and mixed-mode HILIC phases were used in a multilinear regression to determine the contribution of the hydrophobic and hydrogen-bond components to their environmental properties (e.g., bioconcentration, toxicity, sorption to clay minerals, and partitioning to membranes and dissolved organic carbon). Using the current approach, it is possible to predict the environmental behavior of alcohol ethoxylates with only two stationary phases. Retention on liquid chromatographic phases can therefore aid in the understanding of the driving partition mechanisms of alcohol ethoxylates in the environment.

WP057 Quantifying the Equilibrium Partitioning of Substituted Polycyclic Aromatic Hydrocarbons in Aerosols and Clouds using COSMOtherm

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Functional groups attached to polycyclic aromatic hydrocarbons (PAHs) can significantly modify the environmental fate of the parent compound, making it important to assess the behavior of these substituted polycyclic aromatic hydrocarbons (SPAHs) in the environment. However, equilibrium partition coefficients, which are essential for describing a compound's distribution between environmental compartments, are largely unavailable for SPAHs. In this study, COSMOtherm, a quantum chemical software is used to estimate atmospherically relevant partition coefficients for naphthalene, phenanthrene, benz(a)anthracene and benzo(a)pyrene and 66 of their substituted counterparts. The resulting partition coefficients between the gas phase, the aqueous bulk phase, the water surface and the water insoluble organic phase, as well as the salting-out coefficients, served as input parameters for the calculation of phase partitioning of these compounds in atmospheric aerosols and clouds. The predictions showed the influence of compound properties (number of rings, nature of attached functional group) and environmental conditions (size of aerosol/cloud, liquid water content, salting-out effect) on the atmospheric phase distribution of SPAHs. While highly polar substituents such as hydroxyl, nitro and dione are expected to significantly influence the partitioning properties of the parent compounds, the non-polar alkyl substituents showed much less of an effect.

WP058 Development and Validation of Biota-Sediment Accumulation Factors for the Lower 8.3 Miles of the Lower Passaic River using Log-linear Regression

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The Lower Passaic River, part of the Diamond Alkali Superfund Site, is a highly impacted tidal urban estuary located in northern New Jersey that has been the focus of intense study, leading to the recent Record of Decision (Mar-2016) by the USEPA for the lower 8.3 miles. An important component of this investigation was the development of quantifiable site-specific relationships between sediment and fish tissue concentrations for the major contaminants, including 2,3,7,8-TCDD, PCBs, DDT, dieldrin, PAHs, copper, lead and mercury. The data for the analyses came from the Superfund investigation as well as NYC harbor studies including CARP and NOAA RE-MAP programs. These relationships were developed for 3 fish and one invertebrate species, representing a range of trophic levels. For organic compounds, the BSAFs were developed based on lipid-normalized and organic carbon-normalized concentrations in fish and sediment, consistent with EPA guidance. For metals, only sediment concentrations were normalized, to iron content. Concentrations in both media often spanned orders of magnitude, with sample-specific BSAF and BAF estimates apparently varying with sediment concentration. To address this non-linearity, the data were fit with a log-linear regression of the form: C_{fish} = $?_0$+ $?_1$*Ln [C_{sed}/f_n]+ $?_2$Ln(f_{Lipid})where f_n is the fraction of organic carbon or fraction of iron in the sediments, for organics and metals, respectively. The $?_i$ are coefficients in the regression. Thef_{Lipid} term was excluded for metals. This formula reverts to the standard BSAF when the $?_i$ equal 1. Individual tissue concentrations were matched to area-weighted average surface sediment concentrations based on animal home range. The regressions achieved statistically significant relationships for most species-contaminant pairs, often spanning orders of magnitude; maximum R^2 was 0.92 for 2,3,7,8-TCDD. Subsequent to the original work described in the 2014 proposed plan, an additional data set collected in 2012 above the head-of-tide on the Passaic was used as a validation data set for the original BSAF regression approach. In 6 of the 8 contaminant-species pairs reviewed, the new data validated the original regression analysis, and confirmed the extrapolation of the 2,3,7,8-TCDD regressions to sediment concentrations 10 times lower than those in the original analysis. This relatively simple but robust empirical approach was used to develop the site-specific sediment remedial goals for the ROD.

WP059 Monitoring Toxicity of Complex Mixtures in the Savannah River using Passive Sampling Devices

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Previous biomarker research measuring the exposure of fish to a range of compounds, including endocrine disrupting compounds and polycyclic aromatic hydrocarbons, produced significant responses at multiple locations along the Savannah River. This justified a broader investigation into the chemical mixture present in the river and the components responsible for the observed biological effects with the use of passive sampling devices (PSDs). PSDs concentrate contaminants and act as biological surrogates, without the metabolism or depuration of living organisms, allowing for detection of dilute analytes that may fluctuate over time. Two PSDs that complement one another, LDPE (low density polyethylene) strips for nonpolar compounds and POCIS (polar organic compound integrated sampler) disks for polar compounds, were deployed in triplicate along the Savannah River to monitor environmental mixtures both temporally and spatially. Samplers were deployed at three river surface water sites around and below the Augusta (GA) area as well as two locations within Hartwell and Strom Thurmond Dams, which create reservoirs in the Savannah River upstream of Augusta. River surface water sampling sites all possessed continuous river monitoring probes maintained by the Phinizy Center for Water Sciences, measuring pH, specific conductance, DO, and temperature. Chemical analyses were performed on concentrated extracts for each site and time weighted average (TWA) concentrations calculated using compound-specific uptake rates and USGS river discharge data. LPDE extracts were analyzed for 16 priority pollutant PAHs using GC-EI/MS and PCB congeners from Aroclor mixtures 1016 and 1254 using GC-ECD. Select pharmaceuticals and personal care products (PPCPs) were extracted from the POCIS disks and analytical methods were developed using UPLC-MS/MS. Differences in surface water mixture compositions were evaluated for differences in season, site characteristics, and usage patterns. Combined PSD extracts were used in small volume bioassays including the YES assay and zebrafish embryo developmental toxicity assay to link the measured chemical pollutants with observed biological effects in the field.

Contaminant Flux Across Environmental Compartments and Implications for Global Distribution

WP060 Prediction of soluble release of PCBs from contaminated sediment using measured sediment pore water distribution coefficients and DOC

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The release of dissolved hydrophobic organic contaminants (HOCs) from contaminated sediments is often a major source to the aquatic environment. However, quantifying the release in specific environments is challenging because the physical and biological processes involved in this release are diverse, variable, or otherwise difficult to characterize in a quantitative way. Here, we develop and evaluate four approaches to predict the soluble release of PCBs from the sediment bed. These models include the processes of bioturbation and transport across the benthic boundary layer (BBL), assess the importance of using measured rather than modeled sediment pore water concentration, and consider the role of dissolved organic carbon (DOC). Results indicate that the mass transfer coefficient is most dependent on the sediment pore water distribution coefficient, which is obtained from the sediment pore water concentration, and variables that affect the transport across the BBL. DOC in the sediment pore water and water column did not greatly affect the mass transfer coefficient. We applied our findings to the Indiana Harbor and Ship Canal (IHSC) of Lake Michigan and show that deeper sediment section from IHSC will significantly increase ($p < 0.05$) the current release of PCBs from the sediment to the above water by a factor of at least 9.

WP061 Predicting the Airborne Polychlorinated Biphenyl Emissions from the Contaminated Waters of New Bedford, Massachusetts

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New Bedford Harbor (NBH) is a 70 km^2 urban estuary in Massachusetts with sediment contaminated with polychlorinated biphenyls (PCBs) and heavy metals. Aroclor 1242 was the primary commercial PCB mixture used in New Bedford until 1971 when Aroclor 1016 became available for use in the manufacture of electronic capacitors. The usage of PCBs in New Bedford peaked at about 1 million kg per year from 1973 to 1975. Due to this contamination, the harbor was placed on the U.S. Environmental Protection Agency (USEPA) federal list of Superfund cleanup sites in 1982 and is considered to be one of the nation's largest PCB contaminated sites. An environmental dredge of sediment in the harbor is ongoing, with plans to contain the sediment in a confined aquatic disposal (CAD) cell located in the lower harbor. USEPA Region I has been monitoring water concentrations of PCBs since 2006 and significant concentrations have been measured ranging from 10 to 10^5 ng L^{-1}. Using these water data, we have predicted gaseous fluxes as well as emissions of PCB congeners from NBH water, on days when water samples were collected, as a function of the meteorological conditions (e.g. wind speed, air and water temperatures) and individual PCB physical-chemical properties (Henry's law constant, internal energy, etc.). For example, gaseous flux of PCBs (expressed as the sum of the 18 NOAA measured PCB congeners) on June 24th, 2009 was 1.05 X 10^6 ng m^{-2} day^{-1} and a total PCB emissions from the upper Harbor, with an area of 550,000 m^2, was 600 g day^{-1}. These predicted values are at least one order of magnitude higher than other known PCB contaminated water systems, such as the Indiana Harbor and Ship Canal (IHSC), East Chicago, Indiana. Our findings indicate that NBH could be considered as one of the largest sources of airborne PCBs, which could not only affect the local PCB airborne concentration, but may impose a regional impact as well.

WP062 Evaluation of Air-Water Exchange of Polychlorinated Biphenyls at the Lower Duwamish Waterway Superfund Site

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Air-water exchange can be an important sink of PCBs in contaminated tidal estuaries. To determine the flux of PCBs across the air-water interface at the Lower Duwamish Superfund site, passive samplers were deployed in the surface waters and in the air above the water to measure the freely-dissolved and truly gaseous PCB concentrations. The error introduced by the use of passive sampling and several transfer velocity relationships found in the literature are evaluated. Di- through octa-chlorobiphenyls were detected in both the air and water, and the flux was from the water to the air even considering the uncertainty. Although the average flux of 67 ng PCBs/m^2/day is comparable to the flux calculated at other contaminated sites, it proved to not be a major sink of PCB contamination for the Lower Duwamish Waterway.

WP063 Air-water Exchange and Health Risk of PAHs and OPAHs at a Superfund Mega-site

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Chemical fate is a concern at environmentally contaminated sites, but characterizing that fate can be difficult. An important component of characterizing fate is identifying and quantifying the movement of chemicals at the air-water interface. Superfund sites are often suspected sources of air pollution due to legacy sediment and water contamination. A quantitative assessment of polycyclic aromatic hydrocarbon (PAH) and oxygenated PAH (OPAH) diffusive flux in a river system that contains a Superfund Mega-site, and passes through residential, urban and agricultural land, has not been reported before. Here, passive sampling devices (PSD) were used to measure 60 polycyclic aromatic hydrocarbons (PAHs) and 22 oxygenated PAHs (OPAHs) in the air and water. Human health risk associated with inhalation of vapor phase PAHs and dermal exposure to PAHs in water were assessed. Excess lifetime cancer risk estimates show potential increased risk at sites within and in close proximity to a Superfund Mega-site. Specifically, estimated excess lifetime cancer risk associated with inhaling PAHs was above 1 in 1 million within the Superfund Mega-site. We show the majority of PAHs and some OPAHs in the water are from airborne sources. The majority of PAHs detected in both environmental compartments, 16 out of 26, the direction of mass transfer at all five study sites was deposition. This suggests that the river water in this Superfund site is predominantly a sink for airborne PAHs and OPAHs, rather than a source.

WP064 Measurement of anthropogenic and natural polybrominated diphenyl ethers on atmospheric particles: Potential for long-range transport & health effects

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The usage of polybrominated diphenyl ethers (PBDE) as fire retardants in furniture and electronics have become a health concern. These species are known to act as endocrine disruptors. Recently, natural PBDE analogues produced from phytoplankton, blue mussels and sponges have been discovered which may contribute to a greater PBDE pool. Methoxy- and hydroxy-PBDEs found in marine environments are identifiable among other environmental PBDEs and may have more significant health impacts than anthropogenic PBDEs. Previous work has assumed the impact of natural PBDE analogues is limited to ocean environments; however PBDEs may also be transported into the atmosphere through the formation on marine aerosols. Hence, long range transport may also be possible through the atmosphere, leading to exposure far from the ocean. The atmospheric fate and potential health impacts of these species depends on the aerosol size on which they are found. Size-selected marine aerosols were collected with a nano-Micro Orifice Uniform Deposition Impactor (nano-MOUDI) setup from a rooftop atmospheric site in St. John's, NL. Aerosols ranging from 10 nm to 18 um in diameter were sampled over a year to capture any impacts of seasonal phytoplankton blooms. Samples were solvent extracted and analyzed using gas chromatography coupled to mass spectrometry (GC-MS). Temporal trends will be discussed, as well as implications of aerosol concentrations to human exposure and long-range transport for both anthropogenic and natural PBDEs.

WP065 Bioaccumulation of Substituted Diphenylamine Antioxidants and Benzotriazole UV Stabilizers in an Urban Creek in Canada

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Substituted diphenylamine antioxidants (SDPAs) and benzotriazole UV stabilizers (BZT-UVs), previously under reported classes of organic contaminants, were determined in sediment, water, and freshwater biota in an urban creek in Canada. SDPAs and BZT-UVs were frequently detected in all matrices including upstream of the urban area in a rural agricultural/woodlot region, suggesting a ubiquitous presence and bioaccumulation of these emerging contaminants. Spatial comparisons were characterized by higher levels of SDPAs downstream compared with the upstream, implying a possible influence of the urban activities on the antioxidant contamination in the sampling area. In sediment, 4,4'-bis(α,α-dimethylbenzyl)-diphenylamine (diAMS), dioctyl-diphenylamine (C8C8), and dinonyl-diphenylamine (C9C9) were the most dominant congeners of SDPAs, with concentrations up to 191 ng/g (dry weight, d.w.). Benthic invertebrates (crayfish (Orcoescties spp.)) had larger body burdens of SDPAs and BZT-UVs compared to pelagic fish (hornyhead chub (Nocomis biguttatus) and common shiner (Luxilus cornutus)) in the creek and partitioning coefficients demonstrated that sediment was the major reservoir of these contaminants. This is the first report of bioaccumulation and partitioning behaviors of SDPAs and BZT-UVs in freshwater environments.

Assessing Contaminant Effects in Multi-Stress Ecosystems

WP066 Integrated presentation of ecological risk from multiple stressors

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The lack of ecological realism is widely recognised as a limitation in current prospective environmental risk assessment (ERA), as is the failure of making variability and uncertainty explicit and transparent. While these aspects are currently covered by assessment factors, our growing understanding of the chemical, biochemical, and ecological interactions governing adverse effects have the potential to enable next generation ERA to explicitly account for complex interactions. A step toward capturing more ecology and relevance is the use of environmental scenarios that represent biotic and abiotic parameters required to characterise direct and indirect exposure as well as effect and recovery of species. Prevalence plots area new way of presenting ecotoxicological data whilst accounting also for multiple stressors and ecologically relevant parameters. They provide an indication of the maximum population-relevant impact of an effect of interest and the prevalence of this impact. Essentially they answer two related questions: How strong is the effect? In how many locations will we see the effect? We discuss some of the challenges and opportunities involved in bringing these new concepts into everyday risk assessment. One of the key questions revolves around understanding and definition of the protection goal in specific environmental scenarios, and indeed whether certain scenarios require specific modified protection goals. Once the specific protection goal has been established, a metric to suit both the specific environmental scenario and protection goal needs to be defined and agreed. The selection of this endpoint must be carefully considered as different options will lead to different interpretation. We present a framework to integrate probabilistic approaches with mechanistic effect models to assess variable stressors and environmental scenarios. We present a hypothetical case study risk assessment and illustrate the potential benefits of the framework. To do so, we use an individual based model integrating a dynamic energy budget model to assess the potential impact of chemicals associated with local environmental characteristics. We then illustrate on potential policy makers' decisions of the maximal ecologically acceptable impact and the maximal prevalence of this impact. This new framework has the potential to better present ecologically relevant risk by using integrated biological endpoints and to aid more transparent risk communication.

WP067 Driver-response relationships in multi-stressor watersheds: findings from a long-term, integrated assessment of four US streams

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To understand the effects of anthropogenic stressors in multi-stressor environments, managers must consider biota patterns in the context of natural environmental conditions (e.g. flow, temperature), point- and non-point discharges, and taxa-and end-point specific responses (i.e. physiological biomarkers vs. assemblage-level metrics). These assessments benefit from an integrated, weight-of-evidence framework that considers effluent toxicity, exposure relationships, and in-stream response in the context of spatial and temporal variation. Measurements made during a long-term receiving water study (LTRWS) assessed effluent toxicity, and examined patterns relative to non-point source run-off, and point-source inputs from tributaries and pulp and paper mill effluent discharge. Evaluations included effluent toxicity (Whole Effluent Toxicity (WET) tests, life cycle studies), and habitat and water quality relationships to biota (fish, macroinvertebrates, periphyton diatoms and chlorophyll a [chl a]) from multiple sites (n=5-7) in four streams (Codorus Creek, PA; Leaf River, MS; McKenzie and Willamette Rivers, OR). In all streams, most measured water quality parameters were variable across seasons and years, although annual patterns were generally consistent across sites. Statistically significant increases in some parameters (COD, color, conductivity, hardness) were seen at sites downstream of the effluent discharges relative to upstream sites, but at all four study streams some tributary stream parameters were similar or substantially greater than those at main channel sites regardless of effluent exposure. In WET tests (n>100) using effluent from the mills on each of the four streams, fathead minnow growth and Ceriodaphnia dubia reproduction was typically unaffected in 100% effluent exposures. Fathead minnow life cycle tests examining effluent-exposed fish at key life stages showed that eggs/female/day was the most sensitive endpoint measured, with effects not seen until 3-40x the instream effluent concentrations. In the larger rivers (Leaf, McKenzie, Willamette), fish, macroinvertebrate, and periphyton assemblage patterns were driven more by seasonal and annual variation than by differences among sites, while spatial patterns were more prevalent in Codorus Creek biota. The integrated, multi-facetted study design of the LTRWS enables driver-response relationships to be evaluated to disentangle the effects of in-stream stressors and guide management decisions.

WP068 Assessment of seasonal occurrence and risks of antibiotic residues in multi-impacted surface water and groundwater of Jianghan Plain, central China

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Jianghan Plain is one of the major regions of aquaculture production in China, meanwhile, antibiotics as therapeutic and growth promoters have been widely used in the aquaculture and animal husbandry. In this study, four categories antibiotics (macrolides, tetracyclines, fluoroquinolones and sulfonamides) were detected over three seasons in the aquatic environment at Jianghan Plain, central China. Anthropogenic activities

including fishery and livestock breeding were highly concentrated in the study area, and both of manure and fertilizer were used for crop planting. Besides, the shallow groundwater table and frequent precipitation derived intensive interaction between surface water and groundwater. The detection of target compounds in water samples were conducted by using the high performance liquid chromatography-tandem mass spectrometry. The average concentrations of total antibiotics in groundwater samples were with the value of 130.98, 20.27 and 33.65 ng/L in the spring, summer and winter sampling campaigns respectively, and that for surface water samples were with the value of 2.15, 0.98 and 0.71 µg/L in the foregoing three seasons respectively. The results suggested that the highest accumulation of detected antibiotics in the aquatic environment were occurred in spring, and the lowest concentrations of antibiotics were detected in summer and winter in groundwater samples and surface water samples respectively. Norfloxacin as the most predominant compound in groundwater samples, with concentration values ranging from 2.78 to 142.47 ng/L in spring, from 1.19 to 10.76 ng/L in summer and from 4.65 to 45.04 ng/L in winter. The concentrations of erythromycin accounted for the great proportion of the total antibiotic residues in surface water samples, ranging from 0.57-2.91µg/L (spring), 0.08-1.18 µg/L (summer) and 0.03-1.05 µg/L (winter). The method of risk quotient was used to assess the ecological risk posed by target compounds in both groundwater and surface water, and aquatic organisms in three different trophic levels (algae, daphnids and fish) were chosen to conduct the risk assessment. The results of assessment indicated that the overall order of susceptibility was algae > daphnids > fish, and during all of the measured antibiotics, erythromycin and ciprofloxacin were found to be the most harmful to the algae in surface water and groundwater respectively.

WP069 Assessment of immunotoxicity in fish based on the chemical effects on a natural host-pathogen interaction

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In order to assess chemically-induced immunosuppressive effects in fish, we have developed a test method for evaluation of the chemical effects on a natural host-pathogen interaction. As host, we selected common carp (Cyprinus carpio), a species well studied in fish immune system, and tested pathogenicity of three bacterial species, Aeromonas hydrophila, A. salmonicida, and A. veronii against carp. An experimental infection of A. salmonicida by immersion caused high mortality in carp without any additional stressor, while bath infection of others did not show pathogenicity; therefore we chose A. salmonicida as a natural pathogen. In a preliminary test, 90% mortality was observed after 9 days post-infection, when fish were exposed to 1.0 x 10^6 colony forming unit (CFU)/mL A. salmonicida for 1 h. Based on these results, we decided to infect A. salmonicida at < 1.0 x 10^6 CFU/mL, when analyzing the chemical effects on the host-pathogen interaction. For validation of the test method, we used an immunosuppressive agent, dexamethasone as a positive control. Carp were exposed to 1 mg/L dexamethasone for whole experimental period, and fish in unexposed groups were received only 0.01% acetone. One week after the exposure test started, fish from each group were bath infected in 20 L water containing 2.9 x 10^3 (low) or 2.9 x 10^4 (high) CFU/mL A. salmonicida overnight. Unexposed and uninfected fish were set as control. After 7 days post infection, fish in the dexamethasone-exposed and high dose of A. salmonicida infected group started to die, and 40% mortality was observed in the group after 21 days post infection. The dexamethasone-exposed and low dose of A. salmonicida infected group showed 10% mortality after 13 days post infection. On the other hand, bacterial infection-associated mortality was not observed in A. salmonicida infected fish without dexamethasone exposure. These results clearly indicated that exposure to high concentration of dexamethasone suppressed the immune system of common carp, and caused subsequent mortality. Thus, the test method introduced in the present study is thought to be useful to evaluate chemically-induced immunomodulatory or immunosuppressive effects in fish.

WP070 In situ exposures in an agricultural area in Southern Brazil promote osmoregulatory disturbances in the freshwater fish Prochilodus lineatus

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Aquatic ecosystems in agricultural areas may be affected by trace elements such as metals, as they are present in the composition of many fertilizers and fungicides. In areas with a historical and intensive agricultural production, metals may accumulate in the environment over time and represent severe threats to water bodies. Among metal effects, the disruption of osmoregulatory processes is well known. Thus, the objective of this work was to assess osmoregulatory parameters in fish Prochilodus lineatus subjected to in situ tests in an agricultural area in northern Paraná (Southern Brazil) and evaluate organism responses over time. Juvenile fish were confined in large cages (6000 L) at two different sites: the control group (CTR, n = 500) was confined at a fish farming station; the experimental group (EXP) was confined in a lake located inside an area of intense agricultural activity. During the caging period fish remained in contact with the sediment, so they could feed the substrate, but commercial food was also supplied. After 5, 15, 30, 60, 90 and 120 days of exposure a number of fish (n = 50) was removed from the cages and immediately transported to the laboratory where sampling took place. The gills and kidneys were removed for the determination of the activities of Na^+K^+- ATPase (NKA), H^+-ATPase (HA), and carbonic anhydrase (CA) activity, and plasma was collected for the determination of ions (Na^+, K^+, Cl^-, Ca^{2+} and Mg^+). Metal concentrations (Cu, Pb, Cr, Mn, Ni, Zn, Cd and Al) were determined in sediment samples collected in both sites, along the exposure period. In general, EXP site showed higher concentrations of Cu, Ni, Zn and Cd, in comparison to CTR site. When compared to CTR group, EXP fish showed a significant decrease in the branchial NKA activity, after 120 days of exposure, while in the kidney, an increased activity of this enzyme was observed after 60 and 120 days. The activity of renal HA increased significantly after 60, 90 and 120 days of confinement. Changes in the concentrations of certain ions were also seen, with significant reductions of Ca^{2+}, after 90 and 120 days, and Na^+, after 60 and 90 days; conversely, Mg^+ showed a significant increase after 90 days. These results show the in situ exposure of P. lineatus in an agricultural area changed osmoregulatory processes in different ways, and the metals present at these locations are the potential cause of these changes.

WP071 Toxicity and fitness costs in PAH-resistant and non-resistant Fundulus heteroclitus exposed to creosote-contaminated sediment extract and hypoxia

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Chronic creosote pollution at sites along the Elizabeth River (VA, USA) has resulted in the evolution of toxicant resistance in populations of Atlantic killifish (Fundulus heteroclitus) inhabiting those areas. Specifically, these fish are resistant to the acute toxicity induced by exposures to polycyclic aromatic hydrocarbons (PAHs), the primary components of creosote. We have found that a population of killifish that resides at the site Republic, which contains approximately 113,886 ng PAH/g dry sediment (Vogelbein & Unger, 2008), is recalcitrant to both in ovo CYP1A induction and developmental deformities after exposures to simple and complex PAH mixtures compared to the reference site Kings Creek (York River, VA, USA). This adaptation is protective against the acute lethality and teratogenesis associated with PAH exposures. However, we have found that there are trade-offs of PAH-resistance. Our goals for this study were to evaluate how multiple stressor exposures affect wild-caught populations of killifish and how evolved resistance against PAH toxicity influences the tolerance of fish populations to other environmental stressors. To do so, we exposed killifish embryos starting at 24hpf to non-teratogenic doses of a sediment extract that contains a complex, real-world mixture of PAHs along with diurnal hypoxia, an environmental condition common to the Chesapeake Bay and many other estuaries in the United States. Preliminary data in other fish species suggests that this type of co-exposure results in more-than-additive toxicity in developing embryos. Endpoints to be tested further include mortality, hatch rates, gross morphological deformities, CYP1A activity, embryo bioenergetics, and oxidative stress analyses. Our work identified bioenergetic costs of PAH-related adaptations, evidenced by reduced active metabolic rates and subsequent reduced aerobic scopes (both $p < 0.05$) in Republic F1 juveniles and an increased basal oxygen consumption rate Republic F1 embryos throughout development ($p < 0.05$). These fitness costs and others could result in an increased vulnerability of Republic killifish to additional anthropogenic or environmental stressors. This work was supported by NIEHS T32-ES021432 and the Superfund Research Program P42ES010356.

WP072 An ecotoxicological approach to oil sands

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The oil sands deposits in northern Alberta (Canada) are one of the world's largest oil reserves that have unique geochemical, ecological and eco-toxicological characteristics to address. A key issue in the oil sands region is the discrimination of natural versus anthropogenic sources of oil sands-related contamination of surface and groundwater and the resulting cumulative effects on aquatic ecosystem structure and function. Since the the lower Athabasca River and many of its major tributaries flow through natural bitumen deposits, the challenge is to distinguish between the types and levels of hydrocarbon-associated contaminants that occur naturally from those arising from anthropogenic activities related to oil sands mining and subsequent upgrading of oil. The main objective of this study was to evaluate the acute and chronic ecotoxicological effects associated with the slumping of river bank material that is comprised of oil sands deposit that naturally enters the river systems through fluvial geomorphological processes. Ecotoxicological tests were conducted using parental geological material collected from the river banks near the oil exploitation area (Alberta, Canada), in 4 different locations. Samples differ each other mainly in terms of texture and proportional bitumen content due to the differences in the specific geological formations in each river basin. The solid samples (treated as soils), sediment and water-based exposure tests were carried out, using standardized ecotoxicological tests with representative terrestrial (Folsomia candida), sediment dwelling (Chironomus riparius) and aquatic (Daphnia magna, Physa acuta) organisms. Preliminary results confirmed that a suite of methodologies were needed to derive an accurate assessment of possible ecotoxicological and related environmental effects. The use of both soil and aquatic organisms provided complementary knowledge on the possible ecotoxicological effects related to exposure to oil sands materials arising from bank erosion-related processes. Different patterns of toxicity were observed based on the location of the different samples' collection areas and types of slumping material (i.e., % content of bitumen), suggesting that the geological context of the area must be taken into consideration when assessing both reach-specific and regional ecological effects.

Global Non-Point Source Aquatic Contamination Mitigation Strategies: Success and Failures

WP073 An Integrated Vegetated Ditch System Reduces Chlorpyrifos Loading in Agricultural Runoff

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Agriculture runoff containing toxic concentrations of the organophosphate pesticide chlorpyrifos has led to impaired water body listings and Total Maximum Daily Load restrictions in California's central coast watersheds. Chlorpyrifos use is now tightly regulated by the Central Coast Regional Water Quality Control Board. This study evaluated treatments designed to reduce chlorpyrifos in agricultural runoff. Initial trials evaluated the efficacy of three different drainage ditch installations individually: compost filters, granulated activated carbon filters, and native grasses in a vegetated ditch. Treatments were compared to bare ditch controls, and experiments were conducted with simulated runoff spiked with chlorpyrifos at a 1.9 L/s flow rate. Chlorpyrifos concentrations and toxicity to Ceriodaphnia dubia were measured at the input and output of the system. Input concentrations of chlorpyrifos ranged from 858 ng/L to 2840 ng/L. Carbon filters and vegetation provided the greatest load reduction of chlorpyrifos (99% and 90%, respectively). Toxicity was completely removed in only one of the carbon trials. A second set of trials evaluated an integrated approach combining all three treatments. Three trials were conducted each at 3.2 L/s and 6.3 L/s flow rates at input concentrations ranging from 282 ng/L to 973 ng/L. Chlorpyrifos loadings were reduced by an average of 98% at the low flow rate and 94% at the high flow rate. Final chlorpyrifos concentrations ranged from non-detect (< 50 ng/L) to 82 ng/L. Toxicity to C. dubia was eliminated in three of the six integrated trials. Modeling of the ditch and its components informed design alterations that are intended to eventually remove up to 100% of pesticides and sediment. Future work includes investigating the adsorption capacity of granulated activated carbon, costs associated with carbon disposal, and real-world field trials to further reduce model uncertainties and confirm design optimization. Trials with more water-soluble pesticides such as neonicotinoids are also recommended.

WP074 An Overview of Risk Mitigation of Agricultural Pesticides in the Lourens River, South Africa

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The aquatic fate, transport, risk and mitigation of pesticides have been intensively studied in the catchment of the Lourens River (Western Cape, South Africa). The catchment supports intensive deciduous fruit and vineyard production and runoff and spray drift are the most important routes of entry of pesticides into the aquatic ecosystem, with short-term peak concentrations resulting in deleterious effects on macroinvertebrate community assemblages. The importance of runoff and spray drift as a source of contamination is highly dependent on spatial scale with spray drift being more important at a field scale, and runoff being more important at a larger catchment scale. A major focus in the catchment has been to characterise landscape features that influence contamination with an emphasis on identifying suitable mitigation options. Wetlands constructed in tributaries and drainage canals are highly effective in reducing runoff and spray drift associated particle bound and aqueous dissolved pesticide concentrations entering the mainstream Lourens River. These reductions in concentrations have also resulted in reductions in toxicity to aquatic macroinvertebrates. Aquatic macrophytes in tributaries and drainage canals are effective in reducing the downstream transport of spray drift associated pesticide concentrations. The effectiveness of these

in-stream measures is however highly dependent on prevailing hydrological conditions, as runoff associated pesticide concentrations were not as effectively reduced due to increased flow velocity. Furthermore, emergent in-stream aquatic vegetation acted as an effective physical barrier, intercepting spray drift and reducing in-stream deposition of pesticides. While many of the tributaries in the catchment have a relatively wide vegetated buffer strip adjacent to orchards, intensive surveys of the catchment revealed that in almost all cases, preferential flow paths led to the development of erosion rills, providing an unhindered route of entry for runoff into water bodies. The results obtained from the research conducted in the Lourens River have direct implications for the global risk assessment and mitigation of pesticides. Regulation of pesticides in South Africa is however not well developed and research results are discussed within this context, with the aim of providing future research and development opportunities relevant to both South Africa and the rest of the world.

WP075 Mitigation of fungicide pollution in detention ponds and vegetated ditches within a vine growing area in Germany

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Large amounts of fungicides are commonly applied to agricultural fields, particularly vineyards. Following their application, fungicides may accidentally be introduced into agricultural surface waters posing a potential risk for the integrity of aquatic ecosystems. In this context, the present study characterized the aquatic fungicide exposure at base flow and during rainfall-related runoff events in viticulture in Southern Palatinate (SW-Germany) between 2006 and 2009. The mitigation performance of three vegetated ditches (VD) and five vegetated detention ponds (DP) was assessed. The measurements uncovered the presence of four to eleven different fungicide compounds in each of the 81 samples. During runoff events, the ecotoxicological potential – expressed as the sum of toxic units calculated based on the acute toxicity towards algae, Daphnia and fish– of some of the mixtures detected at the inlet of the VD or DP exceeded the Uniform Principle threshold set by the European Union. Both the VD and the DP systems reduced the median fungicide concentrations and thus their associated ecotoxicological potential by 56% and 38%, respectively. This fungicide mitigation efficiency was mainly explained by the plant density and size-related properties of the vegetated systems. Although VP and DP are promising tools to mitigate fungicide exposure, a better mechanistic understanding of the factors triggering the remediation potential finally feeding back into policy decision making is required.

WP076 Non-Point Source Aquatic Contamination Mitigation Strategies: A Global Perspective

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With increased global demand for fuel and food production, a major intrinsic cost may be damage to downstream aquatic ecosystems due to non-point source contamination. Implementation of natural landscape features or bioengineered structures are part of the solution in reducing toxicological or biological effects caused by potential upstream contamination. The aim of this presentation is to highlight what mitigation strategies are in place, working or failing, to reduce concentrations of pesticides and nutrients in receiving waterbodies. This main focus will be to highlight some of the best management practices that are currently being implemented in different locations around the world. The latter portion of the presentation will focus on what approaches are being applied in southern Ontario, Canada to reduce loadings in Lake Ontario and Lake Erie.

WP077 Potential use of Japanese Sweetflag (Acorus gramenius) for Remediation of Contaminated Surface Water

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Acetaminophen, carbamazepine, sulfamethoxazole, and estradiol are often detected in wastewater treatment effluent and surface waters receiving wastewater and reclaimed water. Conventional remediation techniques are often impractical for non-point sources and for compounds with no water quality regulations. One possible remediation technique under evaluation is the use of floating islands established with ornamental wetland plants. Mass balance studies were conducted to characterize the potential uptake of 14C-labelled acetaminophen, carbamazepine, sulfamethoxazole, and estradiol by the ornamental wetland plant, Acorus gramenius (Japanese Sweetflag). Over a 14 day exposure period, there was a 20% reduction of atrazine, 26% reduction of carbamazepine, 40% reduction of perfluorooctanoic acid (PFOA), 60% reduction of sulfamethoxazole, 60% reduction of estradiol, and 100% reduction of acetaminophen. Preliminary results suggest that A. gramenius may be a good candidate for removing acetaminophen, PFOA, carbamazepine, sulfamethoxazole, and estradiol from contaminated surface water. Future research will evaluate the uptake potential of other contaminants with differing hydrophobicity to identify optimal properties for uptake.

WP078 Reducing nutrients and organic micropollutants in a rural wastewater effluent with subsurface filtration treatment technology

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To better understand the efficacy of wastewater treatment in rural Canada, we characterized the occurrence of wastewater contaminants (i.e. pharmaceuticals and nutrients) from lagoon to release at a treatment facility in Dunnottar, Manitoba. Wastewater treatment in this community is performed by the use of a two-lagoon system with subsequent subsurface filtration and ultraviolet treatment. Grab samples were collected during the summer of 2015 to measure pharmaceuticals along the wastewater path and Polar Organic Integrative Chemical Samplers (POCIS) were deployed at the input, output and post-UV treatment on the filtration system. Nutrients concentrations were measured at the input and output of the filter. Significant attenuation was observed for nutrients between the input and the output of the subsurface filter (19-42% ammonia; 55-71% total phosphorus). A total of five pharmaceuticals were consistently detected throughout the filter: atenolol, sulfamethoxazole, metoprolol, propranolol and carbamazepine. Of these five, most were mainly removed between the primary and secondary lagoons (56% carbamazepine; 86% metoprolol; 73% sulfamethoxazole), likely due to photolysis and microbial degradation, with little attenuation being observed for these compounds within the filter, which is consistent with previous studies done by our research group at this facility. The detected concentrations at the output of the treatment system were below levels of concern for aquatic life. Our results suggest that the constructed sub-surface treatment filtration system can provide a low-cost and low-maintenance means to reduce nutrient loadings commonly found in wastewaters.

WP079 Savannah River Site's A-01 Constructed Wetland System: A Model for Sustainable Aquatic Risk Mitigation

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In October of 2000, a constructed wetland treatment system began receiving a combination of stormwater and wastewater from the A-01 outfall located at the U.S. Department of Energy's Savannah River Site (SRS) in South Carolina. The constructed wetland treatment system was designed to treat one million gallons per day of stormwater from a 200-acre watershed (42% of total flow) and effluent from research laboratories (58% of total flow) at SRS. The A-01 outfall is an NPDES permitted discharge, and prior to construction of the wetlands, contained copper at levels toxic to aquatic organisms. The conceptual design of the wetland treatment system was developed from pilot mesocosm studies to identify key aspects of wetland function and performance. The pilot studies determined specific design parameters such as physical/chemical characteristics of hydrosoil, appropriate hydraulic retention time, and selection of wetland vegetation effective for copper attenuation. The full-scale constructed wetland system consisted of an upstream retention basin that provided consistent flow via gravity to eight one-acre wetland cells planted with giant bulrush (Schoenoplectus californicus). The A-01 outfall has consistently achieved compliance for copper, mercury, and toxicity since the wetlands came on line 16 years ago. The constructed wetland system has provided numerous research opportunities from conceptual design through long-term operation. Much of the research will be highlighted here. The A-01 constructed wetland system received recognition from the U.S. Department of Energy and U.S. Environmental Protection Agency Region 4 as a model application of sustainable technology, having saved SRS over $60 million over the life of the system.

WP080 When you wish upon a ditch: Twenty years of bioengineering for agroecosystem services

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Throughout history, agricultural drainage ditches have served to move excess water from production acreage to nearby rivers, lakes, and streams. Research, however, has indicated these unique structures are capable of providing vital ecosystem services such as pesticide and nutrient mitigation. Early studies on vegetated drainage ditches in the Mississippi Delta demonstrated their substantial ability to sorb several different pesticides and transfer them out of the water column. These studies led to further confirmation across the United States, culminating in the Natural Resource Conservation Service's California state office recognizing vegetated agricultural drainage ditches as a modified conservation practice (607A). More recent drainage ditch research has focused on denitrification, carbon sequestration, and ditch metabolism involved with nutrient mitigation. Successful pesticide mitigation results will be briefly highlighted before presentation of the challenges faced in nutrient mitigation, along with results from current mesocosm studies showing the capability of Leersia oryzoides (rice cutgrass) to denitrify and immobilize nitrate.

Thinking Outside the Laboratory Box: An Ecological Approach in Tackling Ecotoxicological Problems

WP081 Bifenthrin causes trophic cascades in aquatic food webs and alters subsidies to terrestrial food webs

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Direct and indirect ecological effects of the widely used insecticide bifenthrin on stream ecosystems are largely unknown. To investigate such effects, a 30-d experimental stream test was conducted by exposing macroinvertebrate communities to a single dose of bifenthrin-contaminated sediment; results were interpreted in the context of a regional-scale assessment of Midwestern (USA) streams. At bifenthrin-sediment concentrations previously thought safe for aquatic life, direct effects of exposure included reduced larval macroinvertebrate abundance, richness, and biomass; whereas, indirect effects included a trophic cascade in which periphyton abundance increased after macroinvertebrate scrapers decreased. Adult emergence dynamics and corresponding terrestrial subsidies were altered at all tested bifenthrin concentrations. Extrapolating these results to the regional stream assessment suggests pervasive ecological effects in Midwestern streams with altered emergence dynamics likely in 39% of streams and a trophic cascade in 7% of streams. We provide new evidence that a common pyrethroid can alter aquatic and terrestrial ecosystem function at the regional scale.

WP082 Multi-Stressor Impacts on Fish Energetics: A Comparison between Lab and Field Studies

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Aquatic organisms face numerous stressors simultaneously in their environments. While each of these stressors pose great threats to aquatic life, studying each stressor individually is not sufficient, as these stressors are not independent, but work cumulatively and synergistically to alter aquatic ecosystems. The objective of this study was to compare and contrast the impacts of multi-stressors under lab and field conditions using two fish species, zebrafish (Danio rerio) and rainbow darters (Etheostoma caeruleum). Under lab conditions, the study examined the impacts of chronic exposure to environmentally relevant concentrations of venlafaxine, an antidepressant pharmaceutical contaminant found abundantly in wastewater treatment plant effluents, in combination to elevated water temperatures on zebrafish. Under field conditions, rainbow darters were collected from two sites, upstream and downstream of a wastewater treatment plant, which served as control and contaminated sites respectively. Muscle tissue was sampled from both lab and field species to measure enzyme activity of major metabolic enzymes and metabolites involved in regulating energetics in fishes. Circulating levels of stress hormone, cortisol, were measured from holding water of fishes. Oxygen consumption was measured using respirometers to assess the effects of stressors on standard metabolic rate, active metabolic rate, and aerobic scope, all of which can be used as ecological performance indicators. The results of this study will contribute to the recognized importance of including multi-stressor approach assessments to make predictions regarding the impact of stressors on fish health and abundance.

WP083 Eutrophication and nanopesticide contaminants interact to reduce ecosystem productivity and microbial functioning in a wetland mesocosm experiment

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Engineered nanomaterials have now been considered pollutants of emerging concern for almost a decade. The recent development of nanopesticides is resulting in widespread application of engineered nanoparticles to agricultural fields. Nanopesticides are likely to become increasingly common components of agricultural runoff, with significant potential for environmental impact in downstream ecosystems. We examined the impacts of chronic 0.05 mg L^{-1} weekly addition of an agricultural copper hydroxide nanoparticle ($Cu(OH)_2$-NP) containing fungicide (Kocide 3000) on wetland mesocosms (small ecosystems). Because we anticipate that nanopesticide exposures will often co-occur with fertilizer enrichment, we crossed our nanopesticide exposure treatment with a nutrient enrichment treatment (Oligotrophic or Eutrophic). We hypothesized that resource availability may have a major effect in driving the resistance and resilience of organisms to the potentially toxic effects of nanomaterials, with nutrient rich (eutrophic) ecosystems having a greater capacity for mitigating stress than nutrient poor (oligotrophic) ecosystems, given the elevated nutrient and energy demands of dealing with toxins. Testing this hypothesis necessitates an emphasis on the "eco" part of ecotoxicology with the focus less on mechanisms of toxicity and more on community and ecosystem level endpoints. As such, we assessed the impact of the nanopesticide on: productivity, respiration and biomass of the macrophyte and periphyton; and microbial activities (C, N, P, S enzyme activities, respiration, nitrification, denitrification) in the surficial sediments and periphyton. After 3 months of chronic exposure to the nanopesticide, there have been observable impacts on primary productivity and microbial functioning in the wetland mesocosms, but contrary to our hypothesis they have only been observed in the eutrophic conditions. Significant decreases of macrophyte and periphyton photosynthesis and respiration were observed, as well as 50–70% decrease in periphyton and sediment microbial enzyme activities. These global effects lead to an increase of the buildup of available N and P in the watercolumn and an increase in greenhouse gas emissions (CO_2 and CH_4). Thus this work highlights both the importance of characterizing the impact of nanomaterials in environmentally relevant conditions and in different ecological contexts, such as trophic status.

WP084 Trait-based approach to detect the impact of chemicals on fish in multi-stress condidions

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The impairment of overall ecological status of aquatic ecosystems is generally attributed to multi-stress conditions, mainly hydromorphological alterations, eutrophication, biological invasions and to a certain extent, chemical pollution. Monitoring programs relying on classical taxonomy-based approach typically failed to distinguish between individual stressors, quantify the overall impact of chemical pollution and identify key toxic pollutants, or rather, key type of chemical mixtures which significantly affect aquatic communities and contribute to ecological status impairment. The aim of the present study was to assess the applicability of the trait-based approach in detection and quantification of the impact of the pollutants and their mixtures on fish communities, with the overall objective to contribute to regulatory – driven process of identification of key or river basin specific pollutants and mixtures which is a main focus of EU FP 7 SOLUTIONS project. The study is based on a large dataset acquired during Joint Danube Survey 3 (JDS 3) in 2013 at 32 sampling sites along the River Danube, which includes biological, hydromorphological (CORINE) and large chemical dataset. Fish taxonomical data (132,498 fish specimens, 67 species, 17 families) were converted into six major traits with a number of states. Taxonomical and trait variation of fish communities along the river was checked by correspondence analysis (CA), chemical composition (255 chemicals) at 32 sites was analyzed by principal component analysis (PCA) and ordination techniques, two canonical correspondence analysis (CCA) – PCA coordinates/CORINE scores were used to explore variation of fish taxonomical and trait-based community structure and in the end, fish data (species, traits /states) were correlated with chemical data by general linear model (GLM). Some of the interesting results include: no significant correlation between any species (taxonomical approach) and any chemical or chemical mixture; no significant negative correlation between any trait / state and any chemical or chemical mixture; high positive correlation of non-feeding adults state with a cocktail of ubiquitous chemicals (metals, PAHs) and high positive correlation (established for the subset of 8 sampling sites) of catadromous, rhithral, lithophilic, speleophilic, brood hiders and rheophil species to a cocktail of 11 chemicals typical for sewage waters –nitrogen, pharmaceuticals and personal care products.

WP085 The Qatari Pearl Oyster: Their reproductive biology, in vitro fertilization, sensitivity to local contaminants for environmental management program

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The marine species Pinctada radiata is the most abundant pearl oyster found in Qatari waters; however, little is known about its reproductive biology, ecological importance and life cycle. Oysters filter prodigious volumes of water, capturing particles down to 5 microns in size and ingesting algae, zooplankton, bacteria and detritus. They are therefore exposed to both dissolved and suspended contaminants. Consequently, they are likely to be excellent indicators of potential contaminants in the Arabian Gulf and can be used for risk assessment and monitoring as part of an effective environmental management program. To investigate seasonal variation in gonad maturation a total of 300 oysters were collected from intertidal habitat from March 2014 to May 2016. Upon collection individuals were weighed, shell length and width recorded and sex determined under light microscope. About 30 oysters were selected from different sizes for histological investigations to determine maturation stage of gonads. The rest of the oysters were used for in vitro fertilization and were exposed to widely used reference toxicants and to local contaminants of concern. Another experiment was carried with 120 oysters collected from the same site to determine the optimum diet for best growth. The oysters were kept in a flow-through system. They were divided into three equal groups and were placed every day into a feeding tank for 2 hours. One group was fed with a combination of microalgae and rice powder, the second group was fed the microalgae alone and the third group was fed rice alone. The results showed that there was no correlation between length and width with sex of oysters. Comparing between field and lab-cultured oysters, there were clear differences between the ratios of males to females as well as differences in sexual maturity. Successful fertilization was achieved with gametes obtained from oysters fed with mixtures of algae and rice (80%), rice alone (60%) and microalgae alone (20%). Data from acute and chronic toxicity tests showed that embryo mortality to be dose-dependent and different sensitivity towards various toxicants were obtained. The protocol for successful in vitro culture and fertilization as shown in the present study provides both oyster embryos and adults throughout the year for performing acute and chronic toxicity studies. Future work will focus on using P. radiata for monitoring contaminants of concern in the Arabian Gulf region.

WP086 Individual-based and system dynamic modeling frameworks to explore a complex energetic process in aquatic communities

A. East, Towson Univ / Environmental Sciences; C.J. Salice, Towson Univ / Biological Sciences/ Environmental Science

Advancing the predictive capacity of mathematical models that inform ecological risk assessments necessitates improving the connection between single species toxicity tests and multiple species systems. To this end, models that focus on community level interactions are of particular interest. Specifically, a focus on lower trophic level organisms that function as energetic mobilizers or recyclers would likely improve effectiveness of community models that have typically focused more on predation or competition. A core addition required to expand single species energetic stressor effect models to community levels is the facilitation that occurs due to waste recycling or energetic mobilization by one organism to the benefit of other organisms. In an effort to explore the potential system effects of facilitation, we have constructed two models that mimic experimental aquatic communities of Daphnia magna, Lymnaea stagnalis, generic 'algae,' and lettuce. These are common arrangements of single species toxicity tests, but also represent a potentially common freshwater system of two functionally and behaviorally different invertebrates, a phytoplankton, and a macrophyte. The modelling approaches are (1) an individual-based model centered on Dynamic Energy Budget Theory models of growth and reproduction (DEB-IBM) and (2) a system-dynamics model parameterized by the consumption rate and waste generation of compartments and the corresponding network that defines potential connections of mobilization and recycling for facilitation processes. The DEB-IBM incorporates facilitation processes and their magnitude through a manipulation of the functional response parameter (f) of receiving organisms (D. magna) as a function of output magnitude from providing organisms (L. stagnalis). Both models suggest a wide variety of responses to facilitation, driven not only by the increased complexity of increased connections but also on the magnitude of facilitation. Of particular importance is the observation that facilitation can potentially explain differential toxicity responses in multiple species toxicity tests; this was confirmed in both models under similar scenarios of energetic stressor exposure. Our results suggest that models designed to explain stressor effects at community levels require a framework for inclusion and exploration of facilitating energetic pathways.

WP087 Biofilm in Large-Scale Stream Mesocosms as a Tool to Assess the Efficacy of Advanced Wastewater Treatment Technologies

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An increasing number of studies have shown, with certainty, that current wastewater treatment processes and technologies fail to remove many chemical compounds we use in our everyday lives. Numerous studies have also shown that some of these compounds affect, among others, endocrine function and can cause dramatic changes in the biological communities of receiving ecosystems. Development of advanced wastewater treatment technologies has closely followed this realization and new technologies are more effective at removing these compounds. Increasing pressure on water resources, with water re-use becoming a real need in many places around the world makes progress in this field ever more important. Evaluation of the biological effects of these compounds on receiving ecosystems, however, remains limited by difficulties associated with field studies, such as lack of true replication and difficulty selecting reference conditions (even in up-stream downstream designs), which limits our understanding of the true biological/ecological effects of these compounds in aquatic communities. These limitations become even greater when attempting to assess and compare differential effects, typically in single systems, from effluents of new wastewater treatment technologies. Now, the 12, 320 m artificial streams of the Advancing Canadian Wastewater Assets (ACWA) facility (Calgary, Alberta, Canada) provide a unique platform to answer these questions. Here we present results of biofilm growth in streams that were divided into 4 treatment groups (n=3) representing a negative reference treatment (14 L s^{-1} of adjacent Bow River water) or one of 3 exposure treatments where Bow River water received a 5 % (v/v) influx of either final effluent (positive reference) from the Pine Creek Wastewater treatment plant (where ACWA is embedded), or the same volume of this effluent after treatment via in-line ozonation or reverse osmosis. Structural (biomass, chlorophyll-a and algal community composition) and functional (community respiration, gross primary production, transformation of organic phosphorus and photosynthetic efficiency) endpoints were assessed in the biofilm communities of the streams to evaluate the effect of the positive reference treatment (Pine Creek final effluent) and whether dosing with effluent from advanced treatment technologies led to any observable changes.

Advanced Analytical Methods for Contaminant Discovery

WP088 Application of non-targeted analysis approaches to assess input of organic micropollutants to San Francisco Bay

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Input of emerging contaminants (EC) such as pharmaceuticals, personal care products, pesticides, and consumer/industrial chemicals to San Francisco (SF) Bay through stormwater or wastewater discharge is of concern due to potential impacts on environmental quality and effects on wildlife. Although proactive efforts have been directed toward monitoring for ECs within the Regional Monitoring Program for Water Quality in San Francisco Bay (RMP), there still remains much uncertainty regarding the identity and fate of the most relevant ECs in SF Bay water, along with their source. We have thus conducted a broad spectrum, non-targeted analysis of polar, water-soluble organic micropollutants in SF Bay using high resolution/accurate mass (HR/AM) mass spectrometry approaches, in order to assess occurrence and establish prioritization of ECs for further fate and effects assessment. In 2016, Polar Organic Chemical Integrative Samplers (POCIS) were deployed at three locations in the San Francisco Bay watershed influenced by different contaminant pathways: urban stormwater runoff (San Leandro Bay), agricultural runoff (Napa River), and wastewater effluent (Lower South Bay). These passive samplers were used to provide an integrated, semi-quantitative assessment of pollutants entering the Bay over the course of approximately three weeks. Grab samples were collected before and after deployment to provide a quantitative snapshot of contaminants for comparison. 24-hour composite samples of wastewater effluent were also collected from several wastewater dischargers. Samples were analyzed using HR/AM Orbitrap liquid chromatography high resolution mass spectrometry and resulting data were analyzed using a custom workflow designed for high-fidelity componentization, prioritization, identification, and semi-quantitation of detected molecular features. Compounds identified through this method include, but are not limited to, detergents and other surfactants, pesticide and pharmaceutical breakdown products, and plastic additives. Preliminary results from the non-targeted analyses of SF Bay water will be presented in context of pathways and (where possible) estimated loadings of ECs to the SF Bay ecosystem.

WP089 Exposure Profile Comparison and Screening of Unknown Contaminants Using LC-QTOF: A Cat Hyperthyroidism Study

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Targeted analytical methods using GC- or LC-MS/MS have been widely employed to study the presence of emerging compounds in various matrices. Recently, use of high resolution mass spectrometry (HR-MS) aimed at examining the human exposome has been rapidly growing. In this study we explore the cat exposome via chemical profiles comparison, identify unknown chemicals of potential health concern, and compare the levels of those unknowns in normal and hyperthyroid cats to examine their possible links to the disease. Pet cats share humans' exposure to household contaminants, albeit with certain differences, including metabolic rates. Nevertheless, cats can be sentinels of human exposure, particularly for toddlers with frequent hand-to-mouth activities. Serum samples from 10 San Francisco Bay Area cats (5 hypothyroid and 5 non-hyperthyroid) were analyzed using an Agilent 6550 quadrupole time of flight (QTOF) MS. Using molecular feature extraction (MFE) algorithm, approximately 2500 distinctive molecular features were extracted from all the samples. Further multivariate analysis using the Mass Profile Professional (MPP) software allowed us to identify 131 features that were statistically different between the two groups ($p< 0.05$). Among them, 76 were upregulated and 55 were down regulated in the hyperthyroid group. Using a compiled database on chemicals found in consumer products, groups of antidepressants, pesticides, PPCP, PFASs and others were tentatively identified based on exact mass, isotope distribution, and isotope spacing. Further identification efforts are underway for those unidentified features that have halogenation patterns. This study will help to better understand not only the cat exposome, but possible body burden changes under hyperthyroidism conditions. The views expressed herein are those of the authors and do not necessarily reflect those of the California Department of Toxic Substances Control.

WP090 Identifying Poly- and Perfluoroalkyl Substances (PFAS) Transformation Products in A Wastewater Treatment Plant

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Recently, poly- and perfluoroalkyl substances (PFASs) derived from aqueous film forming foams (AFFF) were measured at relatively high levels in effluent in some San Francisco Bay Area wastewater treatment plants. A follow-up study was commissioned to investigate the fate of PFASs at an airport wastewater treatment plant before, during, and after a major AFFF introduction event. In addition to routine analysis of PFASs by LC-MS/MS, additional transformation products were identified using high resolution quadrupole time of flight mass spectrometry (QTOF/MS). Using a combination of targeted and non-targeted approaches, molecular features that were extracted from the raw total scan chromatography were tentatively identified with compounds from an in-house PFAS database. For compounds without a database match, chemical formulas were generated based on exact mass, isotope distribution, isotope spacing, and retention time (if available). We preliminarily identified the presence of various PFASs in AFFF formulations, such as 6:2 fluorotelomermercaptoalkylamido sulfonate (FtTAoS), 6:2 fluorotelomer sulfonamide alkylbetaine (FTAB), and two transformation products from the biological oxidation of 6:2 FtTAoS. The integrity of the analysis was validated by standardized sample analysis procedures using Agilent MassHunter Qual and Mass Profiler Professional (MPP) software for multivariate analysis, and high match scores (>90) for the assignments. Available isotope labeled and natural PFAS standards were used as positive controls. This is the first study to examine real time, microbially-mediated transformation reactions in a full scale system. The use of unknowns analysis is critical in establishing transformation reactions and their rates because there are only a few commercially available analytical standards that are applicable to the PFASs present in AFFF. The views expressed herein are those of the authors and do not necessarily reflect those of the California Department of Toxic Substances Control.

WP091 Thermal Desorption of Silicone Wristband Passive Sampling Devices to Assess Fuels, Lubricants, and Coolant Exposure

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The coupling of thermal desorption gas chromatography mass spectrometry with silicone wristbands (WB) as passive sampling devices was utilized to assess volatile organic compounds (VOCs), organophosphates, and poly-α-olefins (PAO). Advantages to this methodology include the ability to examine all three of these chemical classes with the use of only one passive sampling device – reducing sampling costs and effectively eliminating the use of organic solvents – during laboratory processing. Standard-infused wristbands were thermally desorbed in a Markes Micro-Chamber/Thermal Extractor (M-CTE 250) onto Markes Tenax (TA) carbograph 5 (TD) tubes and analyzed with a Markes Thermal Desorption (TD) system interfaced to an Agilent 6890N gas chromatograph / 5975B mass spectrometer (GC/MS). This technology enabled the quantitative assessment of 42 VOCs with detection limits ranging from 0.04-1.35 ng/g WB. Three organophosphates, tributyl phosphate, triphenyl phosphate, and tricresyl phosphate, along with the PAO Xceltherm 500M with detection limits of 0.54 ng/g WB and 54.54 ng/g WB respectively.

WP092 Large Volume In-Vial Extraction Gas Chromatography Mass Spectrometry for Suspect Screening of Semi-Volatile Polyfluoroalkyl Substances in Textiles

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Traditional extraction of per- and polyfluoroalkyl substances (PFASs) in consumer products is typically a complex and multi-step process. Reducing the complexity of analysis may reduce the potential loss of the volatile, short-chained fluorotelomer alcohols. For this reason, a simple screening method was optimized for ten volatile classes and 34 individual volatile polyfluorinated chemical species including: fluorotelomer alcohols, acrylates, and methacrylates, and olefins; perfluoroalkyl sulfonamido (N-ethyl and N-methyl) alcohols, acrylates, and methacrylates. Samples were analyzed by gas chromatography mass spectrometry (GC-MS), coupled with concurrent solvent recondensation-large volume splitless injection (CSR-LVSI), to increase method sensitivity. Textiles were prepared by cutting 1x1 cm^2 sections of material and placing them into 1.5 mL autosampler vials with ethyl acetate that were then spiked with stable-isotope labeled internal standards. Vials were then sonicated in a heated water bath (50°C) and analyzed by a GC-CSR-LVSI-MS approach with a 40 µL injection volume that was optimized in preliminary experiments. This methodology was paired with suspect screening to scout for potential homologues within each family of volatile polyfluorinated species. Preliminary data indicated that C4-C7 homologs are present for N-methyl sulfonamidoethanols, while C2-C10 homologs are present for N-ethyl sulfonamidoethanols. The screening methodology is rapid, with the capability of quantifying a large array of volatile polyfluorinated chemicals, with little to no sample preparation and requiring only small sample size.

WP093 What's the bottom line?

S.T. Glassmeyer, USEPA / Office of Research and Development; A. Batt, USEPA / SED; D.W. Kolpin, USGS / Iowa Water Science Center; E.T. Furlong, USGS / National Water Quality Laboratory

Advances in analytical instrumentation have not only increased the number and types of chemicals measured, but reduced the quantitation limits, allowing these chemicals to be detected at progressively lower concentrations in various environmental matrices. Such analytical advancements have been a particular benefit to pharmaceuticals, hormones, personal care products and other contaminants of emerging concern (CECs). Nevertheless, even as specific methods for CECs are being developed concentrations of many CECs are still near or below the threshold of these improved detection limits. The complexity of these analyses is further compounded by the fact that many CECs are so pervasive in that they may be present in the environments in which samples are collected, processed, or analyzed and may contaminate samples. To ensure confidence in data being generated, an integrated suite of quality assurance (QA) procedures and quality control (QC) samples are necessary. These QA procedures and QC samples prevent or address potential for contamination or loss in both the field and the laboratory, and discriminate between field or laboratory introduction of these artifacts. These QC samples include, but are not limited to, field and laboratory blanks, replicate samples, matrix fortified samples, fortified laboratory samples and instrument blanks. This presentation will examine the QA protocols implemented in and QC sample results from a recent national study of drinking water treatment plants across the United States jointly conducted by the USEPA and the USGS. The QA/QC implemented in this project resulted in nearly a third of the measurements demoted to qualitative instead of quantitative detections, and another third completely censored due to contamination or method performance issues. The presentation will also examine how the handling of detections hovering near the threshold of detection influences the data set.

WP094 Identification of Manufactured Nanoparticles in Soil at Trace Levels with Single-Particle Multi-Element ICP-TOF-MS

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Analytical methods to detect, quantify and characterise manufactured nanoparticles (MNP) in soils and surface waters are urgently needed to assess environmental exposure and risk of these materials. The main challenge in the detection of i.e. manufactured CeO_2 NPs in environmental matrices is the presence of natural Ce-containing minerals of similar size range at concentrations several orders of magnitude above expected levels for manufactured CeO_2 NPs. To identify manufactured CeO_2 NPs against the high natural background we take advantage of the different elemental composition and impurities of natural and manufactured Ce-containing NPs. For example, the Ce to La ratio is expected to be relatively stable at about 2:1 in natural environments, while manufactured CeO_2 NPs show a Ce:La ratio of about 4000:1. However, due to the much higher natural Ce-levels compared to manufactured CeO_2 NP concentrations in the environment, the sensitivity gained from the element ratio alone is insufficient to yield any detectable results when performing conventional bulk analysis on soil samples. We therefore propose an assessment of the elemental composition on an individual particle level by using an ICP-TOF-MS instrument.,This new instrument enables the simultaneous measurement of multiple elements at high sensitivity and time resolution. The applicability of this method for the detection of manufactured CeO_2 NPs in complex samples is demonstrated on a series of natural soils spiked with different concentrations of CeO_2 NPs. Our results show, that we can clearly differentiate between Ce- and La-containing natural particles and Ce-only containing engineered nanoparticles using the ICP-TOF-MS in single-particle mode. Furthermore, a more advanced data analysis method based on a Machine Learning approach and using a multi-element analysis, makes it possible to improve our detection by establishing elemental "fingerprints" of natural and engineered Ce-containing NPs based on 30 selected elements. This provides a more reliable analysis, especially for particle signals close to the particle size detection limit. In this way we are able to confidently detect, identify and quantify manufactured CeO_2 NPs at ppb concentration levels on a 70 ppm natural background. The new method represents a breakthrough for the detection of MNPs in complex matrices at environmentally relevant concentrations and can likely be applied to a wide range of other MNPs in the future.

WP095 Hyphenation of field-flow fractionation and single particle ICP-MS for the assessment of number-based particle size distributions at ultratrace levels

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One of the challenges in characterization of complex nanomaterials in the environment is to obtain number based information such as particle concentration and size distribution at environmentally relevant concentrations, typically predicted to be in the ng/L or µg/L range in surface waters. The combination of field-flow fractionation and inductively coupled plasma mass spectrometry (FFF-ICP-MS) has been proven to be a powerful analytical technique for characterization of environmental samples, but it lacks a direct measurement of particle number concentration since the ICP-MS is used in the traditional "bulk" analysis mode. Single particle ICP-MS (spICP-MS) is a relatively new and rapidly advancing analytical technique which can provide number based information for monodisperse metal and metal oxide nanoparticles at ng/L concentrations. This presentation reports direct hyphenation of spICP-MS to both asymmetrical flow FFF and centrifugal FFF systems. The spICP-MS was utilized as an online particle number detector for characterization of gold, silver, silica and titania nanoparticles as well as different mixtures thereof. The hyphenated technique was able to measure the number-based concentrations and size distributions of the nanoparticles by counting and sizing the respective nanoparticle mixtures in one single run. In general, the ability of spICP-MS to discriminate particle pulses from background is diminished when there is a large amount of dissolved analyte present. Dissolved Ag (µg/L levels) was added to a mixture of Ag nanoparticles (ng/L levels) prior to separation by asymmetrical flow FFF. The semi-permeable membrane (10 kDa cut-off) in the FFF channel allowed the removal of dissolved Ag during the focusing step, thereby improving the ability of the coupled spICP-MS detector to discriminate particles from background.

WP096 Venlafaxine and its major degradation products determination in algae samples

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Emerging contaminants are substances that have been reported recently as anthropogenic compounds that cause damage to the environment even at very low concentrations. Several drugs are within that classification, ending up in the environment through industrial and wastewater discharges, or inadequate disposal. Among pharmaceuticals, venlafaxine, an antidepressant from the class of serotonin-norepinephrine reuptake inhibitors, is usually found in wastewater and contaminated natural water bodies. A analytical method based on liquid chromatography coupled to mass spectrometry (LC-MS/MS) was developed for the determination of this compound and its main degradation products in macroalgae samples. Recovery studies were performed using two species of macroalgae (Laminaria digitata and Saccharina latissima). Freeze-dried samples were weighed (5 g) in Falcon tubes. Three different solvents were tested for the extraction, by adding 10 mL of (i) methanol + formiate buffer 10 mmol L^{-1} (1: 1, v / v); (ii) NaOH 0.1 mol L^{-1}; (iii) acetonitrile + citrate buffer 10 mmol L^{-1} (1: 1, v / v). Tissues were disrupted using and ultrasound pointer for 5 min. Clean up of the extract was performed by Oasis HLB cartridge (60 mg, 3 mL). The extracts were analyzed by LC-MS/MS. Venlafaxine showed a linear response between 0.2 to 20 ng mL^{-1} whereas O-desmethylvenlafaxine (ODV) and N-desmethylvenlafaxine (NDV) showed a linear response from 0.01 to 100 ng mL^{-1}. Instrumental limits of detection (LOD) and limits of quantification (LOQ) for all studied contaminants were 0.01 and 0.2 ng mL^{-1}, respectively. Extractions with acetonitrile + citrate buffer showed the best recovery: between 87 and 111% for venlafaxine, 93 and 105% for ODV, and 108-132% for NDV in spiked algae samples at a concentration of 10 ng g-1 dry weight; and between 80 and 110% with fortification level of 40 ng g-1. In order to verify the efficiency of the method, the method will be applied for the analysis of venlafaxine and target metabolites at environmental levels in macroalgae samples.

WP097 Determination of Nitrate and Phosphate Concentration in Sediment of the Lagos Lagoon

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Eutrophication is a state of nourished water body or sediment as the case may be, this has continued to be a major environmental problem. This study was aimed at determining the nitrate and phosphate concentration in the Lagos lagoon, hence the nutrient availability in the sediment. Sediment samples were at six different locations from the lagoon in the months of March, June and August 2015. The physicochemical properties were determined in the laboratory by titrimetry, colorimetry, Phosphates and nitrates were determined spectrophotometrically; pH, 2.78-7.38 (5.79±1.57); organic matter, 0.76-2.96 (1.97±0.64) bio-available Phosphates, 7.85-20.9 mg/kg (12.2±4.34) Nitrates, 0.495-4.227µg/kg (2.71±1.25). High concentrations of Nitrates and Phosphates in the sediment samples from the Lagoon indicated that the waters flowing into the Lagoon were highly polluted, it is highly contaminated and therefore not suitable for drinking purpose in man. In conclusion, this study is baseline data towards future ecological study, setting guidelines and standards for the conservation and management of resources in economically important wetland in Nigeria.

WP098 Determination of gliphosate and its degradation product in surface water

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This work aimed to evaluate the quality of water resources in Maranhao state with respect to the presence of the herbicide most widely used in the world and its major degradation product. The glyphosate and aminomethylphosphonic acid are of great concern pollutants currently just due to the wide use of this pesticide on crops, mechanical weeding in gardens and family production. The research considered also the parameters established by the National Water Agency (ANA), which manages the National Program for Water Quality Assessment (PNQA) and those established in Resolution 357/05 of CONAMA. The collection points established by PNQA were analyzed for the concentration of glyphosate and AMPA, determined by ion chromatography (IC), and amperometric detection. The analysis was performed using Dionex Ion Chromatograph of ICS3000-DUAL, Thermo Scientific, Term Scientific IonPac AS19, 2x250 mm / AG19 2x50 mm; flow 0.35 ml min -1; 30 oC; GMA; injection volume of 50 uL with autosampler; gradient generated in situ by Thermo Scientific Sionex III EGC-KOH cartridge. Observed the results, it was noted that some cromatrogramas detected glyphosate and AMPA, some just left glyphosate, others only AMPA, others neither compounds; so having a divergent results and realizing that some regions the practice of herbicide use is being made and there is no other records.

WP099 Development of method for analysis of 17α-ethinyl estradiol

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Bisphenol-A (BPA) is an extensively used chemical in industrial processes. It can be used as a monomer in the production of polycarbonates, polymers, epoxy resins and unsaturated polyester-styrene resins. BPA is also presented as lining in food and beverage aluminum cans and as dental sealant. The 17α-ethinyl estradiol (EE2) is an estrogen hormone often used in contraceptive pills with very similar effects of BPA. It leads to great potential hormonal disorders capable to cause adverse health effects on animals and humans. This study developed an efficient extraction method to identify and quantify BPA and EE2 chemicals in tadpoles used in ecotoxicological studies. The high-performance liquid chromatography (HPLC) was used for the analysis. Four extraction assays were performed in order to optimize the processes. They had variations according to reports in the literature. Of all tests performed, the most effective had a recovery of 52% and 32% for BPA and EE2, respectively. This test was conducted in four stages using solid-phase dispersion with hexane and liquid-liquid extraction with acetonitrile. The Amphibians that undergone ecotoxicological tests with BPA were analyzed through the developed procedure performed in this study. No animal presented the compound. The test 4 was the best procedure for extraction and recovery of BPA. Despite the complexity of the analyzed samples, the assays T1, T2 and T3 had lower recovery rates for biological samples (< 1%). No procedure tested in this study was efficient for EE2. The solvents and the correct handling of the samples influenced notably the efficiency of extraction, taking into consideration the specificities and interactions in the analyzed matrices. The solvents for extraction of animal tissue were chosen according to intrinsic characteristics (chemical and physical) of the matrix. Biological samples should be analyzed within few hours after exposure of amphibians in order to detect and quantify the presence of the analytes. Furthermore, the optimized extraction method performed in this study may be an efficient tool for monitoring urban sewage and environmental impact assessment.

Canadian Oil Sands: Advancing Science in Chemical and Toxicological Characterization, Reclamation and Monitoring

WP100 The Effects of Diluted Bitumen (dilbit) on the Hatching Success and Embryonic Development of Sockeye Salmon (Oncorhynchus nerka)

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Diluted bitumen (dilbit) is a crude oil blend produced by mixing heavy crudes and/or bitumen with diluents, typically natural gas condensate, naphtha or a mix of other light hydrocarbons. Current (and proposed) pipelines carrying dilbit traverse the coastal watersheds of British Columbia, where Pacific salmon are at risk of exposure. Sockeye salmon (Oncorhynchus nerka) embryos were exposed to 4 concentrations (0, 13.7, 34.7 and 124.5 μg/L) of the dissolved fraction (DF) of a Northern Alberta dilbit blend immediately following fertilization in a flow-through exposure system for 72 d. TPAH concentrations declined with time and the composition of the DF shifted towards larger and more substituted PAHs. The mortality of embryos and alevins were recorded through the exposure. At the end of the exposure, a subset of individuals from each treatment group were sacrificed for deformity and biochemical analysis. Validation of the exposure and biological availability of hydrocarbons were confirmed by a significant induction of CYP1A gene expression. Cumulative mortality was 6.1% higher in embryos exposed to the highest concentration of dilbit compared to controls. The incidence of the four types of deformity examined (skeletal, craniofacial, finfold, and presence of edema) was generally low, and exposure to the DF of dilbit did not significantly affect deformity rates. The time to hatch was significantly delayed (control group reached 50% hatching at 55 d post-fertilization; the two highest dilbit concentrations achieved 50% hatch by 58 d and 59 d post fertilization) in exposed embryos. Total body lipid content and whole body triglyceride concentrations were significantly increased by 2.2- and 2.7-fold, respectively, in the high exposure group. Whole body protein concentrations in the two higher exposure concentration groups were also affected (31.4% and 28.7% lower than controls). These results suggest that exposure to dilbit at low ug/L total PAH concentrations may cause increased mortality, delayed hatching, and altered biochemistry in sockeye salmon embryos.

WP101 The Effects of Diluted Bitumen (dilbit) on Stress Responses, Iono- osmoregulatory and Immunological Performance of Sockeye Salmon (Oncorhynchus nerka)

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Bitumen is a major type of crude oil extracted from the Oil Sands in Alberta, Canada. Current (and proposed pipelines) carrying diluted bitumen (dilbit) traverse the coastal watersheds of British Columbia, potentially putting populations of Pacific salmon at risk of exposure. Juvenile sockeye salmon (Oncorhynchus nerka, age 1+) were exposed both acutely (24 h, 96 h) and sub-chronically (21 d) to 4 concentrations (0, 13.7, 34.7 and 124.5 μg/L) of the dissolved fraction (DF) of a Northern Alberta dilbit blend in a flow-through exposure system; toxicity was assessed using a suite of morphological, physiological and biochemical endpoints. Total PAH concentrations declined with time and the composition of the DF shifted towards larger and more substituted PAHs. Significant induction of liver ethoxyresorufin-O-deethylase (EROD) activity was observed in DF-exposed fish, indicating hydrocarbon bioavailability. Dilbit was not acutely toxic to sockeye at these concentrations. Plasma cortisol concentrations and gill Na^+/K^+-ATPase (NKA) activity were found to be the most sensitive endpoints; NKA activity was significantly reduced at all 3 concentrations of dilbit after 96 h and 21-d exposures. Plasma cortisol in all treatment groups was higher than in control fish at all time points. Plasma

osmolality, [Cl$^-$], and [Na$^+$] were lower in the two high concentration groups at 24 h and continued to decrease with time. Hematocrit decreased significantly in the two high concentrations after 21 d, while [hemoglobin] increased significantly. Plasma glucose was significantly elevated in the two higher concentrations of dilbit at all time points. Liver glycogen content was significantly reduced at 96 h and 21 d at the highest concentration. Fry (8 month) were exposed (24 h and 42 d) to 124.5 µg/L of the DF of dilbit and then challenged with a seawater pathogen, Vibrio (Listonella) anguillarum. Compared to controls, cumulative mortality in exposed fry was 3.8% and 8.7% higher in the 24 h and 42 d exposure groups. This study suggests that acute and subchronic exposure to low concentrations of the DF of dilbit affects the ability of ELS sockeye salmon to maintain ion homeostasis, reduces immunological performance, and causes a significant organismal stress response.

WP102 Effects of Diluted Bitumen on Hematological Parameters in Exposed Juvenile Sockeye Salmon

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Current (and proposed) pipelines carrying diluted bitumen (a crude oil blend produced by mixing heavy crudes and/or bitumen with diluents) traverse the watersheds of British Columbia, potentially putting populations of Pacific salmon at risk of exposure. To mimic exposures realistic to fish in an oil spill scenario, a bitumen blend sourced from Northern Alberta, Canada, was equilibrated in an aqueous mixture; sockeye salmon (Oncorhynchus nerka) were exposed to the dissolved fraction of this mixture acutely (24 h, 96 h) and sub-chronically (21 d). Four concentrations of the diluted bitumen dissolved fraction (dilbit-DF) were used: 0, 13.7, 34.7 and 124.5 µg/L total polycyclic aromatic hydrocarbons ($PAHs_{total}$). These treatment concentrations were not acutely lethal to sockeye as < 1% mortality was observed. Hematological parameters in sockeye salmon whole blood were evaluated, including: erythrocyte, leukocyte, and hemoglobin concentrations; hematocrit; and secondary indices including mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC). At all concentrations and timepoints, erythrocyte concentrations were not significantly altered by dilbit-DF treatment; however, hemoglobin, MCH and MCHC were significantly elevated after 96 h and 21 d in the 34.7 µg/L and 124.5 µg/L $PAHs_{total}$ dilbit-DF treatments. Changes in these parameters indicate erythrocyte hemoglobin became more concentrated, which can occur as a result of lowered blood oxygen levels (increased oxygen demand) and stress. Leukocyte concentrations were significantly decreased in the 34.7 µg/L and 124.5 µg/L $PAHs_{total}$ dilbit-DF treatments after 96 h of exposure. This indicated the potential for reduced immunological performance, which was confirmed in a related pathogen challenge study by increased susceptibility of dilbit-DF exposed sockeye to Vibrio (Listonella) anguillarum. These results show the importance of understanding the effects of diluted bitumen on salmonid immunological performance. Reductions in immune status and alterations in hematological functions could have major consequences on juvenile sockeye salmon viability as individuals or as a population.

WP103 Fluorescence methods for quantitative analysis of oil toxicity test solutions and assessment of diluted bitumen toxicity to fish embryos

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Valid toxicity tests require the measurement of the concentrations of test substances in test solutions, so that toxicity can be expressed as a function of actual exposure concentrations. However, for complex mixtures of hydrophobic compounds such as crude oil, many publications report toxicity only in terms of nominal oil loadings, largely because access to sophisticated analyses by gas chromatography/mass spectrometry (GC-MS) is limited by cost. As a consequence, there is little or no information on the rate and extent of decline in hydrocarbon concentrations during toxicity tests, and toxicity is significantly under-estimated. In tests of the toxicity of conventional crude oils and of diluted bitumens (dilbits), we developed methods to chemically characterize and quantify oil in water by fluorescence spectrometry (FS). The method involves a 50:50 dilution of water samples with ethanol, scanning the sample at excitation and emission wavelengths of 300 nm and 310 to 460 nm respectively, and comparisons of the area under the emission spectrum with a standard curve, after correction for blanks. Compared to analysis of water samples by GC-MS, FS is low cost (~$1/sample), rapid (under 2 minutes/sample), can be done “in-house”, and with a lower detection limit. The FS technique has been used to quantify the amount of oil in water with different oils, including crude oils, heavy fuel oils, and dilbit. Fluorescence analyses show excellent correspondence to other analytical methods, including measurement of total polycyclic aromatic hydrocarbon measurements (TPAH) by GC-MS. In combination with a limited number of analyses by GC-MS, FS can be used for high frequency analysis of test solutions to generate immediate and detailed chemical characterization of hydrocarbon exposures.

WP104 A proposed whole ecosystem study to examine fate, behavior, and toxicity of a diluted bitumen spill in a Canadian boreal lake catchment

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Despite constantly improving safety measures when transporting petroleum products, whether by pipelines, rail, or truck, there will always exist the possibility of accidental releases (spills). In North America, recent reviews by the Royal Society of Canada and the National Academies of Science have highlighted knowledge gaps regarding the fate and behaviour of oil in freshwater environments. These reports also identified the need for ecosystem-level studies to examine the efficacy of first response strategies, clean-up procedures, and the effects of residual oil on the ecosystem following completion of spill recovery efforts. The feasibility of conducting controlled oil release experiments at the Experimental Lakes Area (IISD-ELA) field station in Northwestern Ontario, Canada will be presented and model scenarios for examining responses related to a dilbit (diluted bitumen) release proposed. The authors are soliciting input from the research community regarding, but not limited to, the most significant knowledge gaps to address, experimental design options, and effects endpoint identification as they relate to oil entering boreal ecosystems, aquatic and terrestrial.

WP105 Longitudinal Patterns of Metal Concentrations in Fine Sediments, Surface Water and Aquatic Invertebrates in Tributaries of the Lower Athabasca River

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One of the largest landscape perturbations in Canada is the result of mining the Athabasca Oil Sands Region (AOSR) in northern Alberta, Canada. The oil sands bitumen deposit in the Lower Athabasca River basin is the largest in North America and covers an area of 42,000 km^2, surrounding the city of Fort McMurray. A significant portion of the bitumen in the AOSR occurs within fluvial deposits of the McMurray Formation (McMF) which outcrop in many of the surrounding rivers catchments, creating natural oil seeps into regional watersheds. Heavy and major metals that co-occur with petroleum hydrocarbons have been found in tributary surface water in the lower reaches, where the river flows through the oil sands deposit of the McMF. Recent studies have suggested that the extensive nature of the current oil sands operations could also have potential consequences on water quality and aquatic ecosystem structure and function in regional rivers. In this study, metal concentrations in fine sediments, surface water and aquatic invertebrates were quantified in two tributaries of the Lower Athabasca River using an environmental disturbance gradient sampling design spanning from upper to lower reaches of the catchments. Both catchments have comparable geomorphology but differ in non-point source geochemical inputs as a result of landuse disturbance from nearby land clearing and related oil sands extraction. Results indicate that among-site differences within catchments in fine sediment, surface water and aquatic invertebrate metal concentrations could be attributed primarily to natural variation and gradients in catchment-related geochemical inputs in both rivers. This study highlights the high natural geochemical complexity of tributary ecosystems in the Lower Athabasca River. Furthermore, we emphasize the importance of defining appropriate baseline/reference conditions against which to assess change in environmental endpoints and the need for monitoring designs and related evaluations to discriminate sources of natural variation from potentially confounding effects related to anthropogenic factors.

WP106 Evaluation of toxicity and contaminant concentration of lake sediments from the Peace-Athabasca Delta, downstream of oil sands deposits

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The Peace-Athabasca Delta in northern Alberta, Canada is the largest freshwater inland delta (321,200 ha) in North America with ecological, historical and cultural significance at local, national and international (recognized as UNESCO World Heritage Site) scales. The ecosystem services of the Peace-Athabasca Delta may be threatened by multiple stressors such as oil sands production, climate change and hydroelectric development. A key concern is the potential for contaminants from oil sands to enter the delta by floodwaters from the Athabasca River. Here we evaluate the spatial distribution of contaminants (metals and polycyclic aromatic compounds) in surficial sediments and their toxicity for 60 delta lakes and link results to hydrological pathways. The assessment of toxicity of the delta lake sediments included a Hyalella azteca survival and growth test (14 day) and a fathead minnow (Pimephales promelas) embryo-larval survival, growth and development test (5-7 day). Toxicity tests showed high rates of survival for Hyalella azteca and Pimephales promelas (between 80-100% survival) indicating low sediment toxicity for the majority of lake surficial sediments from the Peace and Athabasca sectors of the delta. Data on toxicity tests and contaminant concentrations for delta lake sediments will be used to identify potentially vulnerable lakes for further study and monitoring, as needed to improve environmental risk assessment associated with the oil sands activity.

WP107 The impact of atmospheric pollutants on regional fresh waters: An assessment of winter-time deposition in the Athabasca oil sands region

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The mining and upgrading of the Athabasca oil sands releases nutrients and trace elements to the atmosphere. Sources of these atmospheric emissions include the open-pit mines themselves, regional upgrading facilities, exposed coke piles, deforested areas, as well as volatilization from tailings ponds, and vehicle emissions. Regional snowpack chemistry reveals high winter-time loading of pollutants, including key limiting nutrients and EPA priority pollutants. Winter atmospheric loads to six tributary watersheds were calculated using measured snowpack loads and interpolation using geospatial software. Atmospheric deposition maps show a bullseye pattern of contaminant deposition for pollutants of concern with the highest loadings between the Muskeg and Steepbank Rivers. Here we present the results from a three-year intensive sampling effort to understand the fate of winter-time pollutant deposition on regional freshwaters. We focused on those pollutants most enriched in snowpack chemistry, relative to background atmospheric deposition (Al, La, Fe, Co, Cr, Cu, C, Mg, Pb, U, PO^{3-}_4, V, Cl, Na, Sn, Ni, Mo, Sr, Be, As). Concentrations of pollutants of concern in stream water were evaluated from intensive monitoring of six tributary streams during the 2013–2015 open-water seasons. Here we will present the results of these analyses and comment on the fate and impact of atmospheric deposition on regional freshwaters.

WP108 One-pot conversion of petroleum-derived carboxylic acids to hydrocarbons to facilitate compound identification and source tracking

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Oil sands process-affected water (OSPW) contains a complex mixture of petroleum-derived organic acids, which, as whole, have been identified as the acutely toxic components of OSPW. Although a number of individual acids have been identified, the molecular structures of the majority of species remain uncharacterized. However, an increased understanding of the molecular composition of these organic acids in OSPW and natural waters is necessary to enable both a greater awareness of their toxicity, sources, environmental fate, and a more accurate assessment of their risk. The objective of this work was to develop methodology for the simple and efficient conversion of mono- and poly-carboxylic acids, hydroxylated acids, and aromatic acids to their hydrocarbon analogues to facilitate characterization by gas chromatography-mass spectrometry (GC-MS). The developed methodology utilizes n-butyl silane in the presence of a Lewis acid catalyst (tris(pentaflurophenyl)borane, $B(C_6F_5)_3$) to reduce the carboxylic acid and alcohol moieties to their corresponding hydrocarbon groups. The "one-pot" reaction was carried out in 5 mL reaction vials and the resulting products were analyzed by GC-MS without further clean-up. The method was developed and optimized using a series of structurally diverse standard compounds chosen to represent acids previously identified in OSPW. No carboxylic acid or alcohol functional groups were detected following the developed procedure, indicating substantial conversion of the acids to hydrocarbons. Conversion yields were compound dependent, but were typically greater than 65%, with losses likely due to volatilization, rather than incomplete conversion. Once optimized, the methodology was applied to characterize commercially available petroleum-derived acids (Refined Merichem, Aldrich, and Acros) and the acid fraction of OSPW. Initial screening and comparison revealed that fingerprints of C_1-C_4 alkylated polyaromatic hydrocarbon (PAH) homologues (naphthalene, phenanthrene, dibenzothiophene, fluorene), and paraffins, differed amongst samples, confirming source specific differences in petroleum-derived acid composition and suggesting that the developed methodology may be applicable to future environmental forensics studies. Future work will investigate fingerprints of other compound classes and incorporate the developed methodology into environmental fate and toxicological studies.

WP109 Deconvolution of the complex environmental mixture: chemical and biological strategies

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Characterization of toxicological profiles of environmental mixtures is challenging due to the complex components. Here we proposed a hybrid platform by combining chemical analyses and affinity based pull down to deconvolute the toxicity and chemical components of environmental mixtures. Using oil sands as a case study, we developed a pull-down assay combined with untargeted chemical analyses (PUCA) to identify the peroxisome proliferator-activated receptor gamma (PPARγ) agonists. A total of 30 ligands were identified in OPSW extracts by PUCA assay. For chemical strategy, using halogenated compounds as a case study of prioritized chemicals, we developed a data-independent precursor isolation and characteristic fragment (DIPIC-Frag) method to identify halogenated compounds in different environmental matrices. e.g., 2,052 brominated and 4,238 iodinated compounds were identified in Lake Michigan sediments; 1,303 brominated disinfection by-products (Br-DBPs) and 1,637 I-DBPs were identified in drinking water samples; 1,008 brominated compounds were identified in house dust. These results revealed numerous unidentified halogenated compounds in environment, which provided a basic mass spectrometric database of prioritized chemicals. Especially, in house dust, by using correlation matrix analysis, we found brominated azo dyes, rather than brominated flame retardants (BFRs), contributed to the majority (85%) of the total brominated compounds in house dust. This study introduced a hybrid platform to deconvolute the environmental mixtures: for chemical strategy (DIPIC-Frag), we could establish and narrow down a list of prioritized chemicals; for biology strategy, the toxic components in complex mixture could be robustly identified.

WP110 A methodology comparison study for quantitative measurement of naphthenic acid in surface water in the Alberta oil sands region

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Naphthenic acids (NAs) occur naturally in Northern Alberta in the oil sands region. It is also found and concentrated in oil sands process-affected water (OSPW), generated from the bitumen extraction process. The quantification and characterization of NAs in surface water for the oil sands region have been challenging especially since NAs are a complex mixture with mostly unknown structures. Water samples from different sources and locations have different statistical profiles. This makes the determination and production of standard reference materials difficult for method development and calibration. A GC-MS method was developed in the 1990s and was used in the NA monitoring program for the oil sands region. With the advance of high resolution mass spectrometry technology, weaknesses and deficiencies of GC-MS have been identified. In a recent expert workshop in Edmonton Alberta, it was agreed that the GC-MS method should be replaced with more suitable technologies, taking advantage of the latest development of analytical instrumentation. At the workshop, a simple NA definition was agreed to by workshop participants – simple carboxylic acids with two oxygen atoms detected using ESI negative-ion mode; R group can be multi-cyclic and aromatic, but does not include heteroatoms, nor any other functionalities. To support the transition to new monitoring methodologies, a comparison study was carried out using the following three methods: Fourier transform infrared spectroscopy (FTIR), high resolution mass spectrometry (HRMS) and GC-MS. Surface water and OSPW samples were used to generate test samples for the comparison study. The performance of the analytical methods was tested using various naphthenic concentrations, extraction and sample preparation processes. The results confirmed the deficiencies of GC-MS method and indicated good correlation between data from FTIR and HRMS. This study also revealed further insight into the complex nature of NA monitoring and will inform the final development of the standard operating procedures for future NA monitoring in Alberta.

WP111 Passive Sampling Advances Knowledge of Water Chemistry in Rivers of Canada's Oil Sands Region

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Passive water quality monitoring was implemented in the oil sands region of Alberta, Canada in 2012 as a part of the Joint Oil Sands Monitoring program. Semi-permeable membrane devices (SPMDs) were deployed in the Athabasca River upstream, within and downstream of the oil sands minable area (OSMA) and mining activities. Polycyclic aromatic compounds (PACs) in water quality grab samples were for the most part below method detection. Although concentrations in water samples were typically orders of magnitude greater than those estimated from SPMDs (e.g., perylene grab = 100 ng/L, SPMD = 0.40 ng/L), this was not always the case (e.g., C4-phenanthrenes/anthracenes grab = 3.4 ng/L, SPMD = 9.3 ng/L). In contrast, PAC detections in SPMD samples had substantially higher frequencies, allowing for PAC profiling and comparisons between sites and over time. Typical of bitumen within the region, SPMDs deployed in the water contained high concentrations of alkylated PACs, dominated by naphthalenes, fluorenes, phenanthrenes/anthracenes, fluoranthenes/pyrenes and dibenzothiophenes. These included measurable concentrations of compounds with 5 and 6 benzenoid rings (e.g., benzofluoranthenes, benzopyrenes, dibenz[a,h]anthracene, perylene, benzo[ghi]perylene and indeno[1,2,3-cd]pyrene], which are identified as priority pollutants by the United States Environmental Protection Agency. Concentrations were highest within the OSMA and in the open water season, decreasing with distance downstream and under-ice. This work shows that long-term water quality monitoring programs that apply SPMDs will acquire information on trace level organic compounds that is of higher spatial and temporal resolution that that generated from conventional water quality grab samples.

WP112 Survey of PAH concentrations and composition in predatory and forage fish in the Fort McMurray oil sands area with comparisons to environmental regime

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The Fort McMurray region is rich in natural sources of polycyclic aromatic hydrocarbons (PAHs) from exposed bitumen beds; anthropogenic sources have increased with oil sands industry expansion. Here we report on results of a broad survey of PAHs (47 compounds) in eight fish species conducted during 2012-2014 fish health investigations in the oil sands area; results are compared with sediment data collected under the Regional Aquatics Monitoring Program and the Joint Oil Sands Monitoring Program. Athabasca River walleye had average fillet ΣPAHs concentrations of 12.4±5.4 ng/g (72.2±14.1% alkylated) with higher liver (75.6±98.9 ng/g; 82.0±14.0% alkylated) concentrations; white sucker fillet (17.0±8.0 ng/g; 89.3±5.1% alkylated) and liver (64.3±61.7 ng/g; 70.6±15.0% alkylated) concentrations were slightly higher. ΣPAHs in lake trout fillet (12.8±4.7 ng/g; 77.6±7.0% alkylated) and liver (68.6±22.2 ng/g; 63.9±14.0% alkylated) and lake whitefish fillet (13.2±5.9 ng/g; 78.6±6.9% alkylated) from Namur Lake also were low. ΣPAHs were higher in forage fish averaging 105.8±33.2 ng/g (68.2±4.8% alkylated) in trout-perch from the Athabasca River, 277.8±267.3 ng/g (75.5±13.4% alkylated) in slimy sculpin from the Steepbank, Firebag, and Dunkirk rivers, and 125.1±21.9 ng/g (63.8±6.22% alkylated) in pearl dace from the Ells River. ΣPAHs in sediments over this study area were substantially higher (4,745±10,652 ng/g) and more alkylated (91.8±5.9%). Fish ΣPAHs were dominated by low-molecular weight PAHs, particularly naphthalenes, fluorenes and biphenyls whereas medium and heavy PAHs such as dibenzothiophenes, fluoranthenes, pyrene and chrysene, common in sediments, were minor contributors. Burbot (liver) from western Lake Athabasca had the highest ΣPAH concentrations (728±534 ng/g; 95.9±8.3% alkylated) and C4-phenathrene dominated, presumably because of their high lipid content. The European Commission has set fish consumption guidelines of 2.0 ng/g for benzo(a)pyrene and 12 ng/g for the sum of PAHs benzo(a) pyrene, benz(a)anthracene, benzo(b)fluoranthene, and chrysene; neither guideline was exceeded for any species, tissue or location. There were no obvious spatial gradients in PAH concentration and composition although the small number of samples (generally 3-4) examined at each location and time and the shortness of the record limited the statistical sensitivity of such investigations. Apart from mercury, there were no obvious gradients in metals concentrations.

WP113 Assessment of Athabasca River tributaries in the oil sands mining region of Alberta, Canada, using in situ exposures of Hyalella azteca

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As part of the Joint Canada-Alberta Oil Sands Monitoring Program, in situ (caged) exposures with the freshwater amphipod, Hyalella azteca, were conducted in Athabasca River tributaries to assess differences between environmental exposures in areas likely influenced by both natural and anthropogenic sources (sites near mining activity) versus areas likely influenced primarily by natural sources alone (sites in the natural deposit but upstream from mining activity). In situ exposures were coordinated with wild fish and benthic community studies, as well as with laboratory exposures using fish and invertebrates, with the intention to link field assessments and laboratory studies conducted at the same sites. Exposures were conducted in the fall (September-October) of 2010, 2012, 2013, and 2014. Amphipods were collected from a wetland within the Athabasca River watershed but outside the area of mining activity, and deployed in cages at 3 sites on the Ells River, 3 sites on the Firebag River, and 4 sites on the Steepbank River. Cages were removed after 2 weeks, and survival and size of amphipods were assessed before freezing amphipods for subsequent analysis of metals bioaccumulation. Survival was greater than 90% for all years and sites, with the exception of 2 sites in 2012 where high water flows resulted in burial of caged amphipods and reduced survival to 85 and 67%. Average size of amphipods at all sites showed less than 10% difference within the same exposure year. Bioaccumulation of 45 metals revealed that although some metals were present at higher concentrations at some sites when compared to amphipods that were not caged, there were no consistent patterns between years for each river. Toxic units were calculated for the 9 metals for which bioaccumulation data associated with amphipod toxicity were available; no consistent trends were observed between years for each river, and the summed toxic units of the 9-metal mixture were 0.6 or less for all years and sites. The results of these in situ exposures indicate that no differences in amphipod survival, size, or bioaccumulation of metals were attributable to natural and/or anthropogenic sources of oil sands chemicals.

WP114 Relationships between parasites and plasma proteins in male white sucker (Catostomus commersonii) from the Athabasca River

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Shotgun proteomics can be used to assess the health of animals, to determine protein biomarkers that are specific to environmental exposures, and also to characterize unique mechanisms of action of contaminants. As part of a larger wild fish health assessment for the Athabasca River under the Joint Oil Sands Monitoring Program, we successfully developed and applied shotgun proteomics to generate protein profiles from plasma of mature male white sucker taken from three sites along the main stem of the Athabasca River in 2011. The study sites were located within the oil sand deposit including a site downstream of Fort McMurray but above the oil sands operations, and two sites downstream of the oil sands extraction facilities. On average, 376 ± 96 proteins were identified in plasma from each location. Gene names corresponding to those identified proteins were analyzed using interactive pathway software (Ingenuity Systems, Inc.) to determine their core functions and to compare the datasets by location, year, and sex. There were 478 proteins identified in plasma from fish across all sampling locations that were related to immunological functions. The following parasites were enumerated in the same fish samples: Diplostomum spp. (eye fluke), Ichthyocotylurus sp. (fluke), Phyllodistomum sp. (bladder fluke), Dactylogyrus sp. (gill flukes), and Polyopisthocotylea gen. sp. (flatworm). Linear discriminant analysis (LDA) was performed on the parasite counts for each species and the count of proteins related to various immunological functions. Principal component analysis (PCA) was performed on the relative expression (total intensity counts) of each protein related to immune functioning for all individuals. Both of the multivariate approaches revealed strong differences (based upon protein expression, function, and parasite counts) among the three sampling locations.

WP115 Development of wood frog (Lithobates sylvaticus) tadpoles in a natural wetland after embryonic exposure to naphthenic acids: Preliminary findings

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Due to their unique life history, amphibians are sensitive models for understanding the toxicity of process waters produced by bitumen extraction from Canadian oil sands. Naphthenic acids (NAs) are one of the most toxic constituents of oil sands process-affected water (OSPW). Our research team is conducting a series of lab and field-based studies to understand the effects of naphthenic acids (extracted from OSPW) on the health of amphibians at different life stages. In this experiment, we assessed whether a naïve population of wood frogs (Lithobates sylvaticus) exposed to NAs during the embryonic stage display abnormal growth and development as tadpoles that could affect their survival in the wild. In April 2016, we collected wood frog egg masses from a wetland at the Queen's University Biological Station in Elgin, Ontario. Egg masses were then randomly assigned to 15 outdoor microcosms containing 10 L of natural pond water. Following a regression-based design (12 treatments, 3 controls), we added NAs to microcosms at nominal concentrations ranging from 1 to 50 mg/L, representing 1 to 100% of NAs typically found in OSPW. After 5 weeks, tadpoles (Gosner stage 25) were transferred to floating cages (35 cm diameter x 60 cm height) in their wetland of origin. Tadpoles are being examined weekly to monitor their growth, development, and survival. Once tadpoles reach Gosner stage 42, metamorphs will be analyzed for gene expression (RNASeq) to identify possible genetic biomarkers from exposure to NAs. Findings from this study will help improve wildlife monitoring programs in Canada's oil sands region. Additionally, the development of biomarkers may help reduce the reliance on traditional vertebrate test methods to monitor for toxicity.

WP116 Impairment vs local adaptation: Physiological scope of forage fish affected by natural and anthropogenic bitumen sources in Alberta oil sands region

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The lower Athabasca River basin of northern Alberta, Canada is home to fish species that reside within bituminous rich deposits of the McMurray geologic formation. Within the same area, the oil sands industry has increasingly developed in the last fifty years. However, the potential and extent of local adaptation of these indigenous fish species to natural and anthropogenic bituminous sources are not well known. Following a reciprocal cross transplant between sites containing natural bitumen and downstream of industry, a 28 day chronic exposure experiment was conducted using a small forage fish, the fathead minnow (Pimephales promelas). Reference fish olfactory acuity in response to social cues such as conspecific alarm cues and taurocholic acid was impaired to levels that were similar to fish downstream of industry after they were transplanted to either the natural bitumen site or the site downstream of industry. Similarly, respiration rates were observed to decrease to levels that were detected in fish that resided in bituminous-toxicant containing waters prior to experimentation. Downstream and natural bitumen fish had lower olfactory acuity and respiration rates than upstream fish which did not change after the chronic exposure at either site. Fish without pre-existing contact with bitumen – natural or otherwise – displayed impairment in olfaction and respiration to levels comparable to fish from within the oil sands region. Biomarker for toxicant exposure increased in both fish populations from bitumen-containing tributaries. Fish from populations inhabiting bitumen-rich environments did not exhibit local adaptation in the measured physiological traits as was previously hypothesized. Physiological scope of fish from natural bitumen and downstream-of-industry waters may be limited in comparison to reference fish without previous bitumen exposure, which may explain the lower survival rates observed in the former. Data in this current study illustrate how differing toxicant loads will affect the ability of a forage fish population to cope with particular environmental stressors. Although no evidence of local adaptation was observed, the differences in physiological responses indicate an effect of oil sands related toxicants on these fathead minnow populations. Understanding that these forage fish populations exhibit a limited physiological scope can inform reclamation and remediation efforts.

WP117 Regulation of cyp1a, cyp3a, abcb1, and abcc2 genes in northern pike (Esox Lucius) population from different locations on the Athabasca River, Canada

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There is evidence that organic chemicals, including naphthenic acids and polycyclic aromatic hydrocarbons (PAHs) that result from extraction of bitumen from oil sands might enter the Athabasca River and its tributaries. Therefore, identification of molecular biomarkers of exposure to these organic chemicals would be useful for monitoring exposure to these chemicals and potential impacts on organisms. Cellular defence against accumulation of toxic xenobiotics includes metabolism by phase I and II enzymes and export of toxicants and their metabolites via ATP-binding cassette (ABC) transporters. In this study, mRNA abundances of genes that encode two phase I enzymes (CYP1A and CYP3A) and two phase III proteins (ABCB1 and ABCC2) were quantified in livers from northern pike (Esox lucius) collected in Spring of 2012 from three locations on the Athabasca River in Alberta near Fort McMurray, Fort McKay, Fort Chipewyan, and two downstream locations (Fort Smith and Fort Resolution) on the Slave River in the Northwest Territories.. Compared to reference sites, expression of CYP1A, CYP3A ABCB1 and ABCC2 were significantly greater in fishes collected from the two sites in the vicinity of Fort McKay, Alberta, which is the site closest to oil sands activities. Correlation analysis was used to determine the relationship between the expression of target genes, concentration of PAHs in bile and location using Pearson's Correlation and principle component analysis (PCA). Concentrations of 2 and 3 ringed PAHs were correlated with expression of CYP1A but concentrations of 4 and 5 ringed PAHs were correlated with expression of CYP3A ABCB1, and ABCC2. Moreover, PCA revealed site-specific differences in both gene expression and PAHs concentrations. While CYP1A is well established as a biomarker for monitoring the exposure to PAHs, quantification of expression of CYP3A, ABCB1 and ABCC2 might be a promising approach to assess exposure to specific classes of PAHs. Additional research is needed to determine whether other chemicals, including organic chemicals in the aqueous phase of OSPW, that might enter the Athabasca river form tailings ponds, contribute to these effects.

WP118 Effect of dissolved organic fractions from OSPW on toxicity of hydrophobic organic compounds to early life-stages of Japanese medaka

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Toxicity of water soluble organic fraction from oil sands process-affected water (OSPW) to aquatic organisms has been studied, but effects of co-exposure to OSPW and hydrophobic organic contaminants such as polycyclic-aromatic hydrocarbons (PAHs), which are an important class of chemicals in tailings ponds used to store OSPW has not been investigated. This study was conducted to determine if dissolved organic compounds (containing mainly basic and neutral compounds) extracted from the aqueous phase of relatively fresh OSPW from Base-Mine Lake (BML-OSPW) or aged OSPW from Pond 9 experimental reclamation pond (P9-OSPW) modulate toxic potency of the model alkyl-PAH, retene to early life-stages of Japanese medaka (Oryzias latipes). The exposure was performed using a partition controlled delivery (PCD) system made of polydimethylsiloxane (PDMS) until day of hatch. Larvae exposed to only BML-OSPW had no significant change in the incidences of pericardial edema compared to control but were significantly greater in larvae exposed only to retene. Expression of CYP1A and incidences of pericardial edema were significantly greater in larvae co-exposed to retene and dissolved organic compounds from BML-OSPW compared to retene alone at 5 × equivalent of dissolved organic compounds from BML-OSPW . However, co-exposure to retene and a 1 × equivalent of dissolved organic compounds from BML-OSPW, and 5 × equivalent of dissolved organic compounds from P9-OSPW were not significant. Although the exposure to 5 × equivalent of dissolved organic compounds from BML-OSPW caused oxidative stress, larvae exposed only to retene or co-exposed to retene and a 5 × equivalent of dissolved organic compounds from BML-OSPW had no significant oxidative stress. These results suggest that water soluble organic fraction from Base Mine Lake might influence toxicity of alkylated-PAHs to early life stages of fishes but this effect would not be expected to occur at current concentrations of OSPW and aging of OSPW can attenuate the observed effects.

WP119 Assessing the toxicity of naphthenic acid mixtures in the clawed frog Silurana (Xenopus) tropicalis embryos

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Naphthenic acids (NAs) are carboxylic acid mixtures considered to be emerging contaminants of concern. They are detected in Canada's Oil Sands Process-affected Water (OSPW) generated during bitumen extraction at concentrations up to 120 mg/L total NAs. The aim of this study was to assess the toxicity and teratogenic effects of commercial NA extracts (NA1 and NA2) and one Acid Extractable Organic (AEO) from OSPW, using Silurana (Xenopus) tropicalis embryos. Following the Frog Embryo Teratogenesis Assay Xenopus (FETAX), Nieuwkoop and Faber stage 9-10 (early organogenesis) embryos were exposed for 96 hours to NA1 and NA2 (11 treatments) to concentrations up to 24 mg/L, and to the AEO extract (13 treatments) to concentrations up to 96 mg/L (n=7 replicates). The nominal concentrations used covered OSPW relevant concentrations. Embryos were photographed for analysis of abnormalities. The estimated lethal toxicity (LC50) was 9 mg/L (R^2=0.99, 95% CI [8, 10]), 12 mg/L (R^2=0.96, 95% CI [12, 13]), and 45 mg/L (R^2=0.97, 95% CI [44, 47]) for NA1, NA2 and the AEO extract, respectively. The main abnormalities observed were smaller size, edema, gut abnormalities and heart abnormalities, and especially evident in the 8, 12 and 48 mg/L groups of the NA1, NA2 and AEO extracts, respectively. The number and severity of the abnormalities increased with increasing NAs concentration in the test solutions. Our results suggest that NAs from commercial sources are significantly ($p< 0.05$) more toxic than those from OSPW. Efforts are underway to identify causative agents in the very complex mixtures of NAs and to understand whether or not effects observed in the lab may occur with NA exposures in native frog species in the wild.

WP120 Identifying the toxic organic components within bitumen-influenced groundwaters

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Surface mining operations in the region surrounding Ft. McMurray, AB are employed where bitumen deposits are relatively shallow. The by-products of surface mining are stored in large tailings ponds, and have been accumulating for decades with increases in oil sands extraction. Investigations have identified the presence of oil sands process-affected water (OSPW) in nearby groundwater due to tailings seepage. However, these same groundwater systems often flow through bitumen deposits, potentially mobilizing water-soluble bitumen components leading to natural bitumen inputs to the Athabasca River watershed. Current research is focussed on chemically profiling natural and anthropogenic bitumen-derived compound influences to groundwaters, and also on toxicity investigations designed to prioritize compound classes of interest for monitoring initiatives. The present study assessed the toxicity of isolated groundwater soluble organic fractions through exposure to two freshwater fish; fathead minnow (Pimephales promelas) and Japanese medaka (Oryzias latipes). Four samples representing natural bitumen only (two samples) and anthropogenic plus natural bitumen (two samples) sources (>100 L each) were fractionated using a preparative solid phase extraction methodology. This protocol produced three fractions of the soluble organics separated using a range in polarity. Both fish bioassays displayed distinct toxicological differences between fractions within sites and between sites. Generally, the fish bioassays results were very similar but distinct from invertebrate bioassays conducted in parallel. Coupled with high resolution chemical characterization data, this study will aid in guiding future comprehensive fractionation studies intent on better elucidating the bioactive components within bitumen-influenced waters. The present study is important for the development of water quality criteria in future water monitoring programs in the Athabasca region.

WP121 Nrf2-mediated Oxidative Stress Responses of Oil Sands Process-Affected Water and Causative Chemicals Identification

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There is evidence that dissolved organic chemicals in OSPW might cause oxidative stress, but definitive studies linking the adverse effect of oxidative stress with corresponding chemicals in OSPW have not been performed. In this study, a reproducible in vitro bioassay was incorporated to effect directed analyses (EDA) approach to identify oxidative chemicals in OSPW. Activation of nuclear receptor erythroid 2-related factor 2 (Nrf2), was 2.9 ± 0.1-fold greater in cells exposed to a total extract (TE) of OSPW. Among the five fractions of OSPW from HLB cartridges, activation of Nrf2 was 2.7 ± 0.1-, 1.2 ± 0.1-, and 1.7 ± 0.1-fold greater in cells exposed to F2, F1 and F5. Further HPLC fractionation of F2 found the Nrf2 activity centered in F8 (1.30-fold, $\rho < 0.001$), F16 (1.34-fold, $\rho < 0.001$) and F25 (1.28-fold, $\rho < 0.001$).The Nrf2 was not activated in cells exposed to either F3 or F4. Untargeted chemical analysis and a semi-quantitative data mining strategy were developed to enhance the performance of EDA approach. Among >30,000 chemicals detected, only 54 chemicals were predicted to be potential causative components by the two-step EDA approach. By use of exact mass and predicted formula, polyoxygenated heteroatomic chemicals, rather than naphthenic acids, were found to be the major causative chemicals for oxidative stress effects in OSPW.

WP122 Transcriptional responses of embryonic zebrafish exposure to raw and ozonated OSPW and recovery post-exposure

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Oil sands process water (OSPW) is water that is stored in tailings containment structures to ensure it can be recycled for various uses including bitumen extraction, material hydro transport, and process cooling. Progressive aquatic reclamation efforts require increasing OSPW quality to permit hydrological integration of the landscape. Ozone treatment is being studied as a potential tool to assist in remediation efforts, as it has been shown to reduce effects such as cardiotoxicity and neurotoxicity to embryonic fish induced by OSPW exposure. This study provides further insight on the potential benefits of ozonation as a remediation technique through toxicity reduction. In this study we exposed zebrafish embryos to both raw and ozone-treated OSPW from 0-7 days post fertilization (dpf). At 7dpf the embryos were transferred to clean water for recovery. Transcript levels were measured immediately after exposure at 7dpf and again at 9dpf to characterize the gene expression changes induced by embryonic OSPW exposure and to monitor recovery post-exposure. OSPW exposure significantly altered the expression levels of certain biotransformation (CYP1a/b) and neurodevelopment (NeuroD) genes. After two days of recovery, the expression levels of these genes were closer to those of the control than those immediately after exposure, which indicates that fish partially recovered from exposure within two days. This study demonstrates for the first time that embryonic fish recover relatively quickly after exposure to OSPW. By studying recovery time post-exposure we can determine the length of time that OSPW-induced effects persist and whether ozonation ameliorates the time required for recovery.

WP123 Airborne polycyclic aromatic hydrocarbons (PAHs) induce the tumor-related p53 pathway in wild double-crested cormorants living in the Great Lakes area

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Polycyclic aromatic hydrocarbons (PAHs) are known and probable carcinogens that are found in oil products and can be emitted into the environment as a by-product through the incomplete combustion of fossil fuels. Studies have shown that exposure to PAHs in the environment can induce both epigenetic toxicity and genotoxicity, but few studies have related PAH exposure to changes in the putative molecular pathways in free ranging wildlife. Previous work has suggested that double-crested cormorants (Phalacrocorax auritus) had a higher incidence of genetic mutations at sites in Hamilton Harbour, ON, Canada, where higher PAH concentrations in the air and sediment occur. The aim of this project was to determine if airborne PAHs further affected cormorants by altering global DNA modification and/or the tumour-suppressing p53 pathway. Liver and lung tissue samples were collected from cormorants at sites in Hamilton Harbour and Lake Erie with differing levels of PAH exposure.

PAHs have been shown to decrease global methylation (5-methylcytosine) in humans and fish, but here we found only a slight increase in 5-methylcytosine in lung tissue of cormorants living at sites with low compared to high PAH exposure. Furthermore, the tumour-suppressing protein (p53) regulates the pathway that conserves genetic stability by activating DNA repair or by inducing the apoptosis, among others, and it is also known to be mutated in over 40% of cancer cases. Our results from real-time RT-PCR show that expression of genes related to DNA repair in both liver and lung tissue were increased with higher exposure to PAHs. Likewise, mRNA levels of genes involved in cellular apoptosis were also increased in the liver, while expression of genes involved in the regulation of p53 decreased in lung tissue at the site with highest PAH exposure. Further western-blot analysis of p53 and p53r2 indicated that post-transcriptional modifications were also affected by exposure to PAHs.Altogether, data suggest that PAHs activate the p53 pathway, which allows birds to cope with increased exposure to mutagens.

Fate and Effects of Metals: Biogeochemical Perspective

WP124 Effect of pH and natural organic matter on heteroaggregation of CeO2 nanoparticles with clay

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Heteroaggregation of nanoparticles and natural clay minerals in aquatic environment plays an important role in determining their environmental fate and mobility. We studied the heteroaggregation of CeO_2 nanoparticles (NPs) with montmorillonite as well as homoaggregation of CeO_2 NPs and montmorillonite under various conditions, including pH and natural organic matter (NOM). Time-resolved dynamic light scattering (TR-DLS) measurements were used to investigate the aggregation kinetics. CeO_2 NPs and montmorillonite had ionic strength (IS) and pH-dependent surface charge. For this reason, the aggregation kinetics of CeO_2 NPs and clay increased in low pH and high IS. At single system, the critical coagulation concentration (CCC) of montmorillnonite increased with increasing pH. At low pH, montmorillonite aggregated due to electrostatic attraction between positive charged clay edge and negative charged clay face. At high pH, both face and edge were negative charge and therefore montmorillonite was more stable than at low pH. On the other hand, the CCC of CeO_2 NPs was lower at pH 6 than at low or high pH. At pH 6, it was close to pH_{pzc} of CeO_2 NPs, and the stability of CeO_2 NPs decreased considerably, resulting in an extensive aggregation. In binary system (montmorillonite-CeO_2 NPs mixture), the CCC of mixture increased with increasing pH. At low pH, the surface charge of CeO_2 NPs was positive while that of montmorillonite was negative. Because there had oppositely charged surface between CeO_2 NPs and clay, the interactions of CeO_2 NPs and montmorillonite could accelerate aggregation. Whereas, at high pH, both CeO_2 NPs and clay had negative charged surface and heteroaggregation was not increased. However the presence of NOM increased stability of CeO_2 NPs irrespective of pH or the presence of clay. Consequently, particularly low aggregation kinetics of mixture was observed even at over 0.1 M NaCl concentrations due to the steric interruption of NOM. This study suggests that these environmental factors should be carefully considered in assessing the environmental impact of metal oxide nanoparticles.

WP125 The role of metal oxide minerals in nickel bioavailability in field-contaminated sediments

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In freshwater ecosystems the bioavailability and toxicity of nickel (Ni) can be modified by its partitioning to metal ligands. In anoxic sediment, reduced sulfur (AVS) is the primary metal ligand, whereas iron (Fe) oxide minerals can be important binding fractions for Ni under oxic conditions. Lotic sediments are often vertically stratified with thin oxic layers overlying anoxic horizons, and the distinct physicochemical conditions in these sediment layers (i.e., redox, pH) modify their metal binding capacity. Current sediment quality criteria consider AVS as the major binding phase for Ni, but have not yet incorporated ligands that are present in oxic sediments. Our research goal is to assess the role of Fe oxides in Ni bioavailability in lotic sediments. In streams exposed to effluent from Ni mining operations in Thompson, MB, Canada, we assessed Ni bioavailability by coupling spatially-explicit sediment geochemistry with the indigenous macroinvertebrate community composition. Benthic macroinvertebrates, intact sediment cores and overlying water samples were collected in two pairs of reference and effluent-exposed sites. Sediment cores were separated into surface (0–2 cm, “oxic”) and deep (2–4 cm, “anoxic”) samples for geochemical characterization. Effluent-impacted sites contained high concentrations of sediment Ni and AVS, though roughly 60% less AVS was observed in surface sediments. Fe oxide mineral concentrations were elevated in surface sediments and bound a substantial proportion of sediment Ni. Redundancy analysis of the invertebrate community showed surface sediment variables significantly explained shifts in community abundances, and indicated that total sediment Ni and amorphous Fe oxide concentrations were the main environmental variables driving community variation. Relative abundance of the dominant mayfly (Ephemeridae) was reduced in sites with greater total Ni, but accounting for Fe oxide-bound Ni greatly decreased intersite variation in effect thresholds between the two mine sites. Our results provide evidence that solid-phase ligands in oxic sediment, most notably Fe oxides, may be playing a critical role in nickel bioavailability.

WP126 Environment monitoring for trace elements in Hawaii Islands using the small Indian mongoose (Herpestes auropunctatus)

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Concentrations of 27trace elements (Li, Mg, Cr, V, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Rb, Sr, Mo, Ag, Cd, Hg, Pb and Bi) in the livers, kidneys, brains, hairs, muscles, stomach contents of the small Indian mongooses inhabiting Hawaii Islands were determined. Cadmium concentration from Macnut orchard was relatively higher than other areas in Hawaii. Although Pb concentrations in the liver and kidney from Hawaii were not significant differences from other countries, the concentration in the brain was observed significantly higher than the others. As the results of area analysis, it found out that Pb levels in the brains of the individuals collected from the firing range were relatively and/or significantly higher than the other areas, moreover, Pb concentration from the firing range showed positive correlations between the concentrations of As and Sb which are included Pb pellets. Median of Pb concentration from the firing range 2.68 $\mu g\ g^{-1}$ WW (min: 0.395 $\mu g\ g^{-1}$ WW -1510 $\mu g\ g^{-1}$ WW). There were three individuals from Ukumehame firing range which exceeded 3.79 $\mu g\ g^{-1}$ WW which was the mean cerebral Pb level in the rats exposed Pb-acetate, and showed the significantly decreases of the number of total erythrocyte, monocytes and neutrophils, and cellular necrosis, diffused oedema and encephalomacia were observed in the rat brain. Furthermore, two fetuses exhibited elevated Pb concentrations than each mother. These results prompted us to express our concern on possible exposure and health effects in the small Indian mongooses and other animals by Pb derived from firing range.

Fate and Effects of Metals in the Environment: Modeling and Interpreting Effects of Metals Mixtures

WP127 It doesn't always add up: A new approach for statistical determination of non-additive toxicity in metal mixtures that have multiple interactions

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Surface waters often contain mixtures of metals that may cause aquatic toxicity. We investigated acute toxicity to Daphnia magna in binary (Cd-Cu, Cd-Ni, Cd-Zn, Cu-Ni, Cu-Zn, Ni-Zn) and ternary (Cd-Cu-Zn, Cd-Cu-Ni, Cd-Ni-Zn) mixtures and demonstrated that the toxicity can be less-than-additive, additive, or more-than-additive, depending on the metals and their concentrations. When metals having non-additive effects are included in a ternary mixture, a complicated combined effect of those interactions can arise. For example, the toxicity of Cd-Cu and Cd-Ni mixtures is, to differing extents, less-than-additive, but the toxicity of Cu-Ni mixtures is generally more-than-additive. Therefore, ternary Cd-Cu-Ni mixtures could exhibit a somewhat complicated combined effect of those three interactions. We present a method to statistically test for additivity that accounts for uncertainty in both the predicted and the observed mixture-toxicity results, when response-addition is assumed. This method uses toxicity data from "titration" experiments, in which the concentration of one metal in the mixture is varied through an exposure series while the concentrations of the other "background" metals are held constant; and then the concentrations of the background metals are sequentially changed in separate experiments. By plotting the ratio (observed mixture mortality/predicted response-additive mixture mortality) versus concentration of the varied metal, the non-additive and/or additive mixture toxicity are visualized across that metal gradient. Using a Monte-Carlo type randomization method, uncertainty associated with the observed mortality in each combination of metal concentrations and uncertainty in predicted response-additive mortality in each of those mixture combinations are concurrently taken into account to then test for non-additivity on a point-by-point basis. The uncertainty in predicted response-additive mortality is computed from the standard deviations of the EC50 and slope of each individual-metal concentration-response curve that was tested concurrently with a given mixture. When feasible, nonlinear regression curves are fit to the "global" mortality ratio versus concentration relationships. To demonstrate this approach, we provide examples of binary and ternary mixtures that exhibit a variety of additive/non-additive toxicity patterns.

WP128 Genotoxicity produced by Fe and Al mixture on liver and gills of the common carp (Cyprinus carpio)

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Unlike many organic pollutants, heavy metals have been identified as the most dangerous in aquatic ecosystems because these are not biodegradable, so they can be accumulated in different aquatic compartments, thus enhancing its toxicity. It has been shown that they can generate oxidative stress, so they may act as genotoxic agents, and in turn cause damage to the fish, reducing their population or promoting their disappearance from a body of water. The objective of this project was to evaluate the genotoxic damage produced by the interaction of Fe and Al in liver and gill of Cyprinus carpio. For this, organisms (juvenile stage) were exposed to Fe (1 ppm) and Al (0.05 ppm) individually and in mixtures, the concentration used was the maximum allowable limit for the protection of aquatic life, established in México (DOF, 1989). The exposure time was 12, 24, 48, 72 and 96 h, after this, the liver and gills extraction was performed with PBS and homogenized. To assess the damage to genetic material, single cell electrophoresis and micronucleus assays were used. The results showed significant differences ($P < 0.001$) at 12 h in liver and gills of organisms exposed to Al and Fe with respect to control group in comet assay and micronucleus test, and a tendency to recover before 48 h for both organs. However, in organisms exposed to the mixture no recovery is observed, and instead the damage remains throughout the study for both organs ($P < 0.005$). In conclusion, aluminum and iron cause damage to genetic material, this being higher in organisms exposed to the mixture of metals in both organs.

WP129 Development of biotic ligand model for prediction of arsenic toxicity to Aliivibrio fischeri: 1. Effects of pH and major ions

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Arsenic is known as one of most hazardous metalloid that could have a toxic effect to organisms. The importance of methodology to assess its toxicity has been emphasized and as a suggestion, biotic ligand model (BLM) was introduced to provide toxicity assessment of arsenic. This study was conducted to develop the BLM to predict arsenic toxicity to luminescent bacteria, Aliivibrio fischeri and aimed to investigate effects of pH and major ions to its toxicity. Also arsenate, a pentavalent inorganic form of arsenic abundant in aquatic and terrestrial systems, was used along with Microtox®, a toxicity testing system, to perform the experiment observing light intensities emitted by A. fischeri before and after the toxicant was injected. When tested arsenate, its major species were hydrogen arsenate ($HAsO_4^{2-}$) and dihydrogen arsenate ($H_2AsO_4^-$) which varied with pH values. As pH increased there were mostly $HAsO_4^{2-}$ ions in the solution and the toxicity also increased. It demonstrates that the change of arsenic species with pH should be considered when modeling and the methodology to incorporate pH effect into the BLM was also discussed in this study. Besides the effect of pH, some cations and anions were also tested in order to analyze their effects on the arsenic toxicity. Increased activities of SO_4^{2-}, HPO_4^{2-}, NO_3^- and HCO_3^- resulted in decreased arsenic toxicity by having competing effect between anions and arsenate ions to the binding site (i.e., biotic ligand) in the following order: $HPO_4^{2-} > SO_4^{2-} > HCO_3^-$. However, cations such as Ca^{2+}, Mg^{2+}, and K^+ did not have significant effects on its toxicity. Overall, the advanced BLM for prediction of arsenate toxicity was proposed to reflect the arsenic species variation, the competition effect of major anions, and the change of strength in competition effect of pH.

WP130 Development of biotic ligand model for prediction of arsenic toxicity to Aliivibrio fischeri: 2. Effects of humic and fulvic acids

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Arsenic is a hazardous metalloid that is generated not only from anthropogenic reasons but also from natural environment such as mineral sources. Pentavalent inorganic arsinic acid (arsenate) is a major species in aquatic and terrestrial environment. As a methodology to assess its toxicity, a biotic ligand model (BLM) can be effectively applied. In this study, effects of humic acid (HA) and fulvic acid (FA) as surrogates of dissolved organic carbon on arsenate toxicity were investigated to develop the accurate and reliable BLM. Toxicity tests were performed using the Microtox® with luminescent bacteria Aliivibrio fischeri. The research studied arsenate toxicity depending on the HA and the FA concentrations, a bridging effect of cations including Ca^{2+}, Mg^{2+}, and K^+, contact time between the HA or FA and arsenate (5 min, 1 h, 1 d, and 7 d), and the effect of pH values. Possible formation of complexes (through the interaction between FA or HA and arsenate) was also observed with high performance liquid chromatography linked to inductively coupled plasma mass spectrometry (HPLC-ICP-MS) instrumentation. The results of changing toxicity will be reflected to the BLM in order to design a model predicting the arsenate

toxicity. By studying the effect of HA and FA on the arsenate toxicity, the BLM is expected to be applied in various sites and provide more accurate toxicity assessment considering the site characteristics.

WP131 Estimating acute toxicity of metal mixtures to Daphnia magna: A multiple evaluation approach

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The simultaneous exposure of aquatic organisms to multiple metals in real-world environments creates a need for increased evaluation of the toxicity of metal mixtures. Due to the lack of systematic approach to determination of multiple metals toxicity in the literature, many hypotheses can be postulated about the potential interactions of metals in both aqueous and biological media. In this regard, interactions among metals and water constituents in the water medium, as well as competitive binding of metals to receptors on biological surfaces can impact the toxic effects. To assess the toxicity of multiple metals, acute toxicity tests of mixtures of metals, including Cd, Cu, Ni, and Zn, to Daphnia magna were performed in this study. The background test medium was modified filtered river water (Otonabee River, Peterborough, ON. Canada) with 3 mg DOC/L. After multiple runs of individual metal exposures, the binary mixtures were assessed using different total toxic units (TU) based on 1TU = EC50 value of individual metal. A complex series of interactions was observed in binary metals exposures. In the binary mixtures of Cd-Cu, Cd-Zn, and Ni-Zn, an antagonism can be observed in low total TUs equal to 0.5; whereas it turned to synergism in higher total TUs of 2. Further, to identify possible synergism or antagonism interactions between metals, a multiple metal toxicity evaluation program was compared to our empirical data.

WP132 From single to metal mixture exposure: Effects on Plationus patulus population dynamics

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Although it is recognized that some heavy metals (Cu, Cr, Ni, and Zn) are essential for life, it is also known that at certain concentrations their presence can cause even death. Many studies have determined toxicity of single metals and some have addressed the interactive effects of multiple elements at diverse concentrations on aquatic populations. We were interested in determining if consequences caused by two or more metals on rotifer population dynamics were due to element interactions or their individual effects. The rotifer, Plationus patulus, a basal freshwater consumer, was exposed to singly to six elements (As, Cr, Cu, Ni, Pb, Zn) and specific metal mixture combinations (As-Ni, As-Pb, Cr-Cu, as well as As-Cr-Cu-Ni-Pb-Zn) at environmentally determined concentrations. Effects on six population parameters (intrinsic rate of increase (r), relative cumulative reproduction, mortality ratio, mictic ratio, fertilization ratio and number of deformed offspring) were assessed. Occurrence of metal interactions was determined by linear regression analyses. Mictic and fertilization ratio as well as mortality were affected during single element exposures, mostly by Cr and Cu (20X higher than control in both cases), Cu (2X>control), and As (2X>control). Significant negative effects due to As-Ni and As-Pb interactions were observed in r and Cr interacted with a combination of all other elements negatively affecting, r and mortality ratio. Although rotifer population dynamics were altered by metal mixtures, the effects produced by mixtures were not always more detrimental than those caused by single metal exposures. Interactions among elements may contribute to the reduced the toxicity of single elements, which may occur in nature, where organisms are exposed to mixtures of pollutants. This research provides insights on effects of metal mixtures on freshwater organisms, which are not always predictable.

From Phosphates to Food Webs: A Tribute to David Schindler's Legacy in Aquatic Sciences

WP133 Will climate change exacerbate pollutant bioaccumulation in marine food webs? Perspectives from an ecosystem modelling approach

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Anthropogenic climate change and chemical pollution are amongst the major stressors on marine ecosystems in the 21st century, posing substantial ecological and human health risks. Understanding the combined effect of climate change and pollutant impacts is crucial for those tasked with managing ecological and human health risks in the long-term. However, the combined effects of these two stressors on marine foodwebs are still not clear. Several studies suggest that climate change is already changing pollutant fate and transport in the atmosphere, and affecting exposure and accumulation of pollutants in marine organisms that can subsequently be affected by adverse health effects. Top predators and humans with high rates of seafood consumption such as marine mammals and First Nations communities in the Northeastern Pacific (Canada and US), may be most vulnerable to such impacts. To test this hypothesis, we developed a trophodynamic model (Ecopath with Ecosim) to examine the impact of climate change and acidification on the bioaccumulation of organic chemical pollutants, including polychlorinated biphenyls (PCBs) and organic mercury (MeHg), in the Salish Sea marine food web in the Northeastern Pacific Ocean. The ecosystem model is driven by projected climate change variables, including temperature, pH, dissolved oxygen and primary production under a 'strong mitigation' (low emission) and 'business-as-usual' (high emission) scenarios (Representative Concentration pathways, or RCP 2.6 and 8.5). The model also tracks and predicts the bioaccumulation of pollutants (PCBs and MeHg) with the Ecotracer module. We then examine the effects of different potential pathways of climate-pollutant interactions on the biodiversity and fisheries in the ecosystems. This study improves our understanding of the interactions of climate and pollution impacts, contributing to a more comprehensive understanding of the risks of multiple human stressors, and highlights key areas for concerted research and potential mitigation policies.

WP134 Validation of in ovo embryo microinjections using selenomethionine to simulate maternal transfer in the fathead minnow (Pimephales promelas)

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Selenium (Se) is a developmental toxicant of increasing concern because it can be released into the aquatic environment in significant amounts from industrial and natural processes. Inorganic Se released into surface water is biotransformed and bioaccumulated by microorganisms and algae as selenomethionine (SeM). SeM is then transferred to higher trophic levels, such as aquatic invertebrates and fish. Early life-stages of fish are highly sensitive to SeM exposure and are primarily exposed via maternal transfer. Developmental deformities (e.g. spinal curvature, craniofacial, edema) might occur as a result of embryo exposure to maternally transferred SeM. However, maternal transfer is difficult to study in native species of concern in Canadian ecosystems, especially for those species that are long-lived or endangered. This study will be the first step in developing an embryo injection model to help interpret maternal transfer of SeM. The fathead minnow was selected as a model organism based on its wide distribution throughout North America and its extensive use in previous regulatory testing and research. Fathead minnow embryos will be injected with graded concentrations of (9, 18, or 36 µg SeM/g d.m – egg) and developmental endpoints will be compared with those from a parallel maternal transfer study. Establishing an embryo injection model for predicting toxicity of SeM through maternal transfer will make testing a broader range of species more feasible. Future research using this model will aim to determine Se sensitivity in early life stages for native species of concern (e.g. white sturgeon and rainbow trout).

Aquatic and Terrestrial Plants in Ecotoxicology and Risk Assessment

WP135 Ability of cupric ion activity to predict growth and survival of individual plants and community-level parameters

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High copper concentrations in soils with depressed pH can be phytotoxic. Cupric ion activity, measured as pCu = $-\log Cu^{2+}$, integrates effects of copper and pH and can be used to evaluate potential phytotoxicity of soils. We developed dose-response curves of pCu against terrestrial plant endpoints measured in a greenhouse (emergence, survival, shoot height, shoot weight, and root length) and field (percent canopy cover, species richness) in semi-arid mining sites to assess if pCu is strongly predictive of phytotoxicity. Covariates were added to the model to account for other soil parameters that affect plant survival and growth, including organic matter, chemistry, and soil type. The laboratory plant species evaluated included the agricultural species, alfalfa, and two species native to the study sites, sideoats grama (Bouteloua curtipendula) and tansyleaf tansyaster (Machaeranthera tanacetifolia). The two native species were further split into seeds collected from the study sites and seeds obtained from nurseries to evaluate if plants growing in mineralized areas have evolved more tolerance to high copper than plants growing in non-mineralized areas. The dose-response model that combined all five seed types evaluated in the greenhouse fit the data well with $R^2 > 0.74$, showing a sigmoid response of reduced plant performance with lower pCu (= higher toxicity). Site-collected seeds of native species were not more tolerant of low pCu than agricultural or nursery acquired seeds when median effect concentrations (EC50) were compared. Soil location type (in bedrock, steep slopes, or level areas with or without topsoil), iron, and clay content of soils were covariates that significantly shifted the dose-response curves as follows: Topsoil present in flat areas and more extractable iron were protective, whereas higher clay content was detrimental to plant growth and survival. A linear model best fit the richness data collected in the field when separated by soil type ($R^2 = 0.83$). The pCu did not predict plant cover well for steep slopes or flat areas lacking topsoil but was predictive for bedrock and flat areas with topsoil. The effect levels were generally consistent between the greenhouse phytotoxicity and field community studies after accounting for natural variability in reference areas without high copper. The results show that pCu is a good predictor of individual and community parameters, better than either copper or pH alone.

WP136 Exposure Assessment Modelling Approach to Non-Target Plants Through Runoff from Agricultural Fields

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The USEPA uses the screening model, TerrPlant, to estimate exposure to non-target terrestrial plants from a single application of pesticide. Audrey III is a higher tier exposure model that has been developed by USEPA to estimate exposure to plants in a Plant Exposure Zone (PEZ). The objective of this study was to investigate the magnitude and likelihood of exposure of non-target plants to pesticide residues through runoff from agricultural field to an adjacent PEZ. TerrPlant and AUDREYIII will be compared to two vegetative filter models: PRZM-Buffer and VFSMOD. PRZM-Buffer is a modified version of the Pesticide Root Zone Model (PRZM), a rainfall-runoff simulation model, to simulate pesticide fate and transport in a PEZ. VFSMOD is a vegetative filter strip (VFS) model designed to simulate VFS processes to remove sediment and pesticides from field runoff/erosion. Current EPA Tier II scenarios for PRZM were used to represent main field simulations. Movement of pesticide through the PEZ and the concentrations for the segments were modeled with the PRZM-Buffer model and VFSMOD. Results from these two models will be compared to each other and to USEPA models TerrPlant and AUDREYIII. Multiple widths of buffers were assessed to determine distance required for soil concentrations to drop below level of concern for non-target crop.

WP137 Glyphosate and Dicamba Inhibit Flowering of Native Willamette Valley Plants

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Successful flowering is essential for reproduction of native plants and production of food for herbivores. It is also an important alternative endpoint for assessment of ecological risks from chemical stressors such as herbicides. We evaluated flowering phenology after herbicide drift treatments for nine native Willamette Valley plant species grown together in small (0.45 x 0.45 m) field plots. Study taxa were: Eriophyllum lanatum (ERLA, Oregon sunshine), Iris tenax (IRTE, toughleaf iris), Prunella vulgaris var. lanceolata (PRVU, lance selfheal), Camassia leichtlinii (CALE, large camas), Festuca roemeri (FERO, Roemer's fescue), Elymus glaucus (ELGL, blue wildrye), Ranunculus occidentalis (RAOC, western buttercup), Fragaria virginiana (FRVI, Virginia strawberry), and Potentilla gracilis (POGR, slender cinquefoil). Research was conducted during the summer growing season at two Oregon State University farms in Corvallis, OR in 2010 and 2011. The effects of glyphosate and dicamba alone, and in combination, were determined for simulated drift rates of 0.01 to 0.2 x field application rates (FAR) of 1119 g ha^{-1}active ingredient (AI) for glyphosate and 560 g ha^{-1} AI for dicamba. Flowering phenology was rated by presence of buds (1), buds turning into flowers (2), flowers (3), flowers deteriorating and seeds beginning to form (4), and mature seeds (5); with data analyzed statistically for treatment (n=8 for each treatment) vs. control plots (n=8). Flowering patterns varied by species, farm and year; but, in general, herbicides alone or in combination reduced the number of plants with flowers on a sampling date and/or delayed the onset of full flowering for 3 species in 2010, and for 6 species in 2011. For example, the number of flowering plants was reduced with dicamba and/or glyphosate at 0.1 x FAR for ERLA and PRVU, with dicamba and/or glyphosate at 0.2 x FAR for CALE, with glyphosate at 0.1 x FAR for IRTE and RAOC, and with glyphosate at 0.2 x FAR for POGR. Results of this study provide evidence that herbicide drift can affect flowering phenology of native plants.

WP138 Is the Tier-1 effect assessment for herbicides protective for aquatic algae and vascular plant communities?

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For the admission of plant protection products on the European market, the environmental risk assessment (ERA) follows a tiered approach. In the first tier of the risk assessment scheme, experiments are required with standard test species. If plant protection products fail the first tier of the risk assessment, higher tier assessments may be applied including microcosm and mesocosm studies. In the aquatic Tier-1 effect assessment for plant protection products with a herbicidal mode of action, algae and/or vascular plants usually trigger the environmental risks. This 1st tier effect assessment includes chronic tests with at least two algae and one macrophyte (usually Lemna). Although considered of a chronic nature based on the duration of the test, the measurement endpoints derived from the laboratory tests with plants and that are used in the 1st tier effect assessment for plant protection products are effect concentrations to 50% of the test organisms (EC_{50}'s). In other European legislative frameworks (e.g. underlying the Water Framework Directive) effect concentrations to 10% of the test organisms (EC_{10}'s) are used. This paper contributes to a validation of the tiered herbicide risk assessment approach by validating the standard first-tier effect assessment with results of microcosm and mesocosm studies. We evaluated the protective value of the Tier-1 effect assessment using EC_{50} and EC_{10} values of standard test algae and macrophytes based on either the growth rate endpoint or the lowest available endpoint for growth rate or biomass/yield. These values were compared to the Regulatory Acceptable Concentrations (RACs) for the threshold option as derived from microcosm and mesocosm studies. A dataset of 14 herbicides was compiled based on regulatory data approved for inclusion in the risk assessment underlying the admission of pesticides in Europe. The Tier-1 RACs based on respectively ErC_{50} (9 cases) and EC_{50} (12 cases) values for algae and macrophytes resulted in 80% and 75% of the cases in a sufficient protection level when compared with the higher-tier RACs derived from microcosms and mesocosms. The Tier-1 RACs based on NOErC/ErC_{10} (6 cases) and NOEC/EC_{10} (7 cases) values were in all cases protective when compared with RACs from microcosms and mesocosms.

WP139 Occurrence and behaviour of emerging organic contaminants in a large-scale constructed wetland in Singapore

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Constructed wetlands are commonly used to simulate the wetland process in a more controlled environment. These nature-assisted water treatment systems may be used as substitutes or supplements for conventional wastewater treatment systems. Constructed wetlands have proven effective for removal of COD, BOD, nutrients and heavy metals. There are relatively few studies on emerging organic contaminants (EOCs) in constructed wetland systems. The objective of the present study is to assess the occurrence, distribution and bioaccumulation behavior of several EOCs in a large-scale constructed wetland in Singapore. This constructed wetland was designed to treat landfill leachate and consists of a pretreatment system (equalization tank, aeration lagoons and sedimentation tank), five constructed reed beds and five polishing ponds. Three plant species are grown within the reed beds, including cattail (Typha angustifolia) vetiver grass (Chrysopogon zizanioides) and papyrus (Cyperus papyrus). The current study focused on assessing the occurrence and behaviour of synthetic musks, triclosan and methyl-triclosan, as well as several halogenated flame retardants. The majority of the target EOCs were detected in the different compartments of the wetland, raw leachate, equalisation tank, sedimentation tank, reed beds and polishing ponds. Polycyclic musks (galaxolide and tonalide) and the chlorinated flame retardant, Dechlorane Plus, exhibited the highest concentations in water and planty samples. The translocation factors (TFs) of the compounds ranged between 0.22 and 7.23. TFs tended to drop for very hydrophobic compounds with log Kow > 5. The root concentration factors (RCFs) and leaf concentration factors (LCFs) varied substantially among chemicals. LCFs were typicall higher than RCFs for these compounds. The results help provide a better understanding of the key determinants and mechanisms governing transport, bioaccumulation and phytoremediation of these contaminants in constructed wetlands.

WP140 The effects of the aquatic herbicide diquat on native and invasive macrophytes

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Invasive aquatic plant species are a significant threat to the health of aquatic ecosystems and demand for chemical control options are likely to increase as invasive species spread and reach nuisance levels. Currently, data on the effects of chronic, ecologically relevant concentrations of current-use aquatic herbicides on non-target biota are lacking. The objective of our study was to assess the effects of the aquatic herbicide diquat on target and non-target biota using outdoor mesocosms (300 L) to simulate natural systems. The experiment consisted of a control and five treatments reflecting environmentally relevant concentrations of diquat, each with five replicates. Biota included a) native and invasive macrophytes collected from nearby waterbodies (Myriophyllum spicatum, Myriophyllum sibiricum, Elodea canadensis and Ceratophyllum demersum), b) natural communities of phytoplankton and periphyton, c) juvenile amphipods (Hyalella azteca) and d) northern leopard frog tadpoles (Lithobates pipiens). The effects of diquat on invasive and native macrophyte communities in semi-natural conditions will be discussed. The relative sensitivity of individual species to diquat when grown in mesocosms will be compared with their sensitivity when grown individually in laboratory conditions. Overall, results from this study will assess the potential risks versus benefits diquat poses to the aquatic environment.

WP141 The impact of nano copper oxide and arsenic on the seeds germination and seedling growth conditions of the rice plant (Oryza Sativa)

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nCuO and As altered seed germination and seedling growth for an important rice species (Oryza Sativa). 1/5th strength of Hoagland solution was used as nutrient. Five treatments of nCuO (0.1, 1.0,10, 50, and 100mg/L) were made with the nutrient solution. As (18 ppm) was added to the sand medium as As treatment. Forty replicates of rice seeds were exposed to each of the following 12 treatments: control, As treatment, five nCuO treatments and five As+nCuO treatments. The seed germination and seedling growth test was proceeded in an incubator for 18 d. The temperature was set at 25 ? during the day (16h), and 20 ? at night (8h). Results showed that: seeds started germinating on day 4; both time and treatment significantly affected the germination rate (time p-value < 0.001, time*treatment p-value =0.007); according to the germination curve, from day 4 to 8, seeds germination was boosted with higher concentration of nCuO (10,50 and 100 mg/L), while those treated with lower concentrations of nCuO and with As showed lower germination rates compared with control; However, on day 18, the germination rate with all nCuO treatments except 0.1mg/L was higher than or equal to the control, while it's higher for As with 1.00mg/L nCuO, and lower for the seeds with the rest of As + nCuO treatments. Moreover, all the seedling length and whole dry biomass were significantly different with different treatment groups($p< 0.05$). As treatments decreased the length and dry weight of both shoots and roots. Lower concentration of nCuO (0.10 mg/L and 1.00 mg/L) alone enhanced the seedling growth with increasing length and dry weight of both shoot and root while the higher concentration of nCuO inhibited the seedling growth. Seedlings growth in As treatments were more inhibited than in treatments receiving As+nCuO. On day 18, the seedling survival rate in the nCuO treatments were consistent with the germination rate rank, whereas the results for As treatments were more consistent with the whole dry biomass. The activity of antioxidant enzymes, superoxide dismutase (SOD) and catalase (CAT), and GSSG/GSH ratio will be measured to compare the oxidative stress caused by different treatments, which will help explain the potential mechanism of the different impacts on the seedling growth conditions. EDS-TEM will be used to identify the distribution and speciation of the chemicals of interests, in order to determine the uptake and transportation of nCuO and As in the seedlings.

Bringing Probabilistic Risk Assessment into Criteria Development

WP142 Application of a Lead Shot Ingestion Model for Waterfowl at a Former Shooting Range Site

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Intentional ingestion of residual lead shot as grit can be a critical exposure pathway for waterfowl species at former shooting range sites. A quantitative understanding of this exposure pathway at a site can support risk and/

or remedial decisions for lead shot. Despite its availability since the early 2000, the general framework for modeling lead shot ingestion by birds has not be widely applied for waterfowl species. At a former shooting range site near a coastal estuary, the ingestion of lead shot from sediment by waterfowl species has been identified to be the exposure pathway of greatest ecological concern. Historically, remediation was performed at this site to remove lead shot from the sediment in the intertidal and shallow subtidal areas, primarily to mitigate this critical exposure pathway for the waterfowl species using the site. Subsequent erosional impacts to the sediment and winnowing effects have resulted in limited sporadic patches of high shot concentration in these remediated areas. A lead shot ingestion model has been applied to estimate the probability that an American black duck (Anas rubripes) will ingest a given number of residual lead shot during its lifetime. The approach, results, and discussion of the waterfowl lead shot ingestion model, including model assumptions, input parameters (receptor-specific and site-specific), and uncertainty evaluations, in the context of on-going and future remedial and/or risk decisions at the site, will be presented.

WP143 Exposure analysis of alkylphenol ethoxylates and their metabolites in US surface waters: combining deterministic and distributional analyses

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C8 and C9 alkylphenol ethoxylates (APnEO, where n = 4-40) are widely used surfactants mainly in a variety of industrial applications since their use in consumer cleaning products has declined over the last decade. In most uses, spent APnEO are sent to wastewater treatment plants for treatment. During treatment, APnEO break down to low-mole AP1,2EO, AP and a variety of low-mole carboxylated APnEC. These degradation intermediates are released via effluent discharges into receiving waters in which they are routinely detected. Due to their aquatic toxicity, the USEPA developed a chronic water quality criteria for freshwater organisms of 6.6 µg/L for the most toxic degradation product, nonylphenol. This criteria was developed using mainly acute toxicity data and acute-to-chronic ratios. Sufficient chronic aquatic toxicity data now exist such that various statistical techniques can be used to assess toxicity including probabilistic, species sensitivity distributions, and distributional analyses. This presentation follows up on an exposure analysis of AP and APnEO in US surface waters that was conducted in 2007 in which the authors compared aggregated concentrations to the USEPA WQC for NP. More recent monitoring data shows some reductions in both environmental occurrence and concentrations of APnEO degradation products. Results of traditional deterministic assessments (hazard quotients) and analyses that compare the distributions of recent measured concentrations and chronic ecotoxicity data are presented and compared to previous findings. The results show that more recent concentrations of AP/AP1,2EO are mostly non-detected in US surface waters, and nearly all aggregated detected concentrations are well below the WQC and lower 5th centile of the chronic toxicity data. The aggregated concentrations that do exceed the WQC are found in streams comprised of nearly 100% effluent.

WP144 Human Inter-individual Versus Population Level Dose-Response Curves Induced by 2,3,7,8-Tetrachlorodibenzo-p-dioxin in Primary B Cells: Linear or Not?

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The effect of inter-individual variability within the human population is not typically assessed in traditional toxicological studies. However, populations within which chemical exposures occur are heterogeneous. Understanding how population dynamics and inter-individual variability impact dose response relationships (DRRs) is critical for accurate risk assessment and protection of human health. A recent report from the National Research Council (NRC) suggests that, when accounting for inter-individual variation in responses, traditionally assumed nonlinear DRRs for non-cancer endpoints would better be explained with a linear model in the low-dose region. While the NRC has acknowledged potential differences between individual and population-level responses, the assumption of low-dose linearity has not been adequately assessed empirically. With the potential to cause undue harm to public health and industrial and municipal finances, such an assumption warrants scientific proof. To address this, we utilized TCDD-induced inhibition of primary human B cells as a model. B cells were isolated from 51 unique human donors and exposed to increasing concentrations of the environmental contaminant 2,3,7,8-tetrachlordibenzo-p-dioxin (TCDD) on a logarithmic scale. Two endpoints were assessed: 1) number of IgM secreting B-cells and 2) amount of IgM secreted. Two approaches were taken to model DRRs. First, EPA's Benchmark Dose (BMD) software was used to assess the DRR between TCDD concentration and average population response at each dose. Second, multilevel modeling was conducted to directly address the issue of individual versus population level DRRs and their corresponding shapes. As such, the model accounts for unequal variances, obviating the need for variance stabilizing transformations of the response. Initial results using BMD indicate that TCDD has a significant suppressive effect in the number of B cells secreting IgM and the concentration of IgM secreted ($p< 0.05$). BMD model comparisons of population-averaged DRRs indicate that the low-dose region is best fit to a nonlinear model, suggesting the presence of a threshold in the population response to TCDD. Multilevel modeling supports these findings, yielding greater clarity regarding the population DRR. Based on initial findings, our study suggests the need to retain an assumption of nonlinearity in the low-dose region of the DRR during risk-management decision-making for non-cancer endpoints.

WP145 Idaho's Experience Using PRA for Human Health Criteria

D. Essig, Idaho DEQ / Water Quality

In March of 2016 Idaho concluded three and one-half years of rulemaking to adopt updated Clean Water Act criteria to protect human health from exposure to toxins through ingestion of water and consumption of fish. Early on in the effort it was decided to pursue probabilistic risk assessment (PRA) to develop the new criteria and a fish consumption survey was mounted with the aim of producing distributions of fish consumption in Idaho. Criteria were proposed based on PRA, but ultimately the criteria adopted were based on traditional deterministic calculations. This presentation will address reasons why Idaho chose to go down the PRA path, bumps along the way, why this path was ultimately abandoned, and some recommendations for smoothing the way for future use of PRA in water quality standards.

WP146 Probabilistic Ecological Risk Assessments – Are they worth your time?

G.I. Greenberg, Gradient Corporation; D.G. Skall, Gradient

Ecological risk assessments evaluate risks to likely receptors exposed to media potentially impacted by site activities. Typically, a deterministic ecological risk assessment (DERA) is conducted for representative receptors with upper-end exposure assumptions (e.g., 100% diet, home ranges equal to site) and central tendency values (e.g., body weight) that ignore the variability of the available data. In a DERA, the inputs for the exposure models and effects concentrations are single values which are used to calculate a simple ratio of effect (i.e., hazard quotient [HQ]). Alternatively, probabilistic ecological risk assessments (PERA) reduce the uncertainties associated with limited data and the variabilities associated with modeling by characterizing risks based on the magnitude of risk as well as the probability of occurrence. We conducted ecological risk assessments for five terrestrial receptors exposed to metals in surface soils, surface water, and associated prey items using both deterministic and probabilistic methods. To simplify the process, the same exposure point concentrations and toxicity reference values were used. The two methods differed in exposure parameters (i.e., body weight, home range, percentage of diet). For the PERA, we also compared two methods of calculating ingestion rates (diet-specific field metabolic rates [FMR] vs. FMRs for representative animal groups). We compared DERA HQs to PERA HQ ranges and determined which input parameters contributed the most to the overall risk results. We determined that in addition to producing more realistic mean HQ values compared to the DERAs, the PERAs can also provide more conservative estimations of risk for receptors with large home ranges. For receptors with small home ranges, the percentage of terrestrial invertebrates in the diet impacted the PERA mean HQs by up to a factor of 4. When comparing PERA HQs using the two different ingestion rates, risks using the diet specific FMRs resulted in lower HQs, but with more variability than HQs using FMRs for representative animal groups. While performing PERAs requires more time, PERA HQs provide information on the magnitude of risk and the probability of occurrence. DERA HQ values only note that the values are conservative, but provide no information on how conservative they are. Consequently, information regarding the probability of occurrence could vastly improve remediation goals and decisions.

WP147 Probabilistic Human Health Water Quality Criteria Calculator

M. Buonanduci, G. Houck, P.D. Anderson, Arcadis US, Inc.

Arcadis has a developed publicly available risk assessment calculator that States and other interested parties can use to derive State-specific human health water quality criteria (HHWQC). The calculator is designed to be transparent but allow States the flexibility to employ the State-specific assumptions that they believe are appropriate. This flexibility allows States to use either point estimates or distributions for any or all of the parameters included in the equations employed by the United States Environmental Protection Agency (EPA), including trophic level-specific fish consumption rates and bioaccumulation factors. In addition to the parameters explicitly listed in the EPA equations, the calculator also includes the ability for users to input distributions for implicit parameters that affect the outcome of the equations but are not listed specifically in the equations (e.g., exposure duration, loss of chemicals from fish tissue during cooking, fraction of fish consumed that are caught within the state, fraction of fish consumed that reside within the water body for their entire life cycle). This presentation will introduce the calculator, which is available on the Idaho Department of Environmental Quality website, and demonstrate how it can be used to derive ambient water quality criteria using probabilistic risk assessment methods. This derivation process uses an iterative approach in which a water concentration is selected and the resulting risk distribution is compared to risk management goal(s). If one or more goals is exceeded, the process is repeated using alternative chemical concentrations until a concentration is identified that results in a risk distribution that meets all risk management goals. That concentration is the HHWQC.

Adverse Effects of Chemicals on the Microbiome

WP148 Analysis of Animas Watershed Sediment Bacterial Communities after the Gold King Mine Breach

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On August 10, 2015 a tailing pond at the Gold King Mine near Silverton, CO was breached when workers attempted to add a tap to aid in restoration efforts. As a result, approximately three million gallons of water containing elevated concentrations of cadmium, lead, arsenic, zinc, copper, iron, beryllium and other toxicants were released into the Animas watershed. Presumably, deposition of metals in sediments along the watershed present a selective pressure for sediment-borne bacteria inhabiting these niches. Here we report on the structure of sediment bacterial communities along the path of the waste outflow from the mine through Cement Creek, the Animas River, and proximal regions of the San Juan River. In the absence of baseline data collected before occurrence of the breach, samples upstream of the confluence of Cement Creek with the Animas River and the confluence of the Animas with the San Juan River were collected to serve as unaffected controls. Analysis of 16S marker genes was used to determine diversity metrics and population structure within the sediment-borne communities and these were compared with communities from unaffected sites. Additionally, abundance of metal resistance determinants was analyzed to garner information regarding how native bacterial communities adapt to mixed metal stressors.

WP149 Evaluating changes in the gastrointestinal microbiome of rainbow trout (Oncorhynchus mykiss) as a consequence of pollutant exposure

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The gastrointestinal microbiome plays a critical role in organism health as it influences metabolic processes, plays a key role in host nutrition, and contributes to intestinal and immune system development in vertebrates, including fish. Many factors can affect the composition of the intestinal microbiome in fish including diet and host phylogeny. While a number of studies have shown that microorganisms are sensitive to low concentrations of pollutants, to date little information is known about the effects of contaminants on the bacterial community composition of the intestines. Any changes in gut microbiome composition have the potential to negatively influence fish health, and due to the essential role of bacteria in the gut, research is needed in this area. To further explore the impacts of contaminants on the intestinal microbiome of fish, this study investigated the potential effects of two pollutants (triclosan and benzo(a)pyrene), representing two chemical classes of environmental concern, namely antimicrobials and polycyclic aromatic hydrocarbons (PAHs) on microbial composition in the gut of rainbow trout. Antimicrobial compounds are extensively used in aquaculture, as well as in humans and animals, to treat bacterial diseases, which can lead to residues entering the aquatic environment and possible subsequent microorganism toxicity. PAHs are ubiquitous in the environment and can elicit a variety of deleterious effects on a wide range of organisms, including bacteria. Gastrointestinal microbiome composition was determined by next generation sequencing of the 16S rRNA gene using an in house Illumina MiSeq system. This research aims to provide insight into novel aspects of toxicity and chemical metabolism in fish, and further our understanding of the important role played by gut flora in host well-being and adaptation to changing environmental conditions. Furthermore, information generated from this study may be used in identification of bioindicator taxa present in the gut microbiome that are sensitive to certain contaminant classes, and development of biomonitoring strategies around known pollutant sources.

WP150 Linking contaminant induced microbiome shifts to host health in fishes

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Host-associated mucosal surfaces (gill and intestine) are important sites for normal development of host immune response, homeostasis, nutrient uptake and overall health. Impacts on a healthy microbiome following exposure to an environmental contaminant are not well-characterized, specifically in non-aquaculture teleosts. Typically, few microbes dominate the limited niches available in healthy tissues. Imbalances in these communities can have detrimental physiological effects. Contaminant exposure may allow rare taxa to compete for resources and dominance. In this study, multiple species of fish were challenged with a known pathogen (Vibrio anguillarum) following exposure to oiled sediment. Using 16S rRNA high-throughput sequencing, short-term microbiome responses were examined in lower gill and intestinal tissue from oil-exposed and pathogen challenged fishes. Two alpha indices, Chao 1 and Shannon, were used to determine changes in species richness and evenness. The gill microbiome was rapidly and significantly altered by oiled exposure;

while no significant difference in alpha diversity metrics were found in intestines (One-way ANOVA, Tukey HSD $p < 0.05$). The gill microbiome from Oil/Pathogen-challenged fish saw the greatest species richness and evenness compared to No Oil/No Pathogen-challenge exposures. Oil exposure resulted in Alcanivorax (a known PAH degrader) and Shewanella (implicated in fish spoilage), dominating the gill microbiome. A complete loss of members of the Photobacterium, occurred in both oil-exposed intestine and gill tissues. Lower levels of contaminant exposure induced significant changes to the gill microbiome, demonstrating an immediate sensitivity of gill fauna to environmental toxicant challenge, suggesting an important link between environmental contamination, microbiome function and host health.

WP151 Phthalates in the gastrointestinal system: Impacts on host-microbiome function

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Phthalate esters, a class of chemicals used as plasticizers in polyvinyl chloride products such toys, water bottles, and medical supplies, are ubiquitous in aquatic environments. These chemicals are also classified as obesogens, or chemicals thought to be involved in metabolic disorders like obesity. However, the mechanisms by which phthalates affect metabolic function are largely unknown. During diet and waterborne exposures, the gastrointestinal system acts as a first line of defense, and consequently may be impacted by phthalate contaminants. As a result, the delicate balance of host-microbiota function may be disrupted. Alterations in the gut microbiome and host gastrointestinal system may disrupt nutrient processing, which can result in downstream phenotypic impacts. In this study, we examine whether low-level chronic exposure to a diethyl-hexyl phthalate (DEHP) alters the microbiome and gastrointestinal function of zebrafish (ZF). ZF were separated into three treatments: (1) a control group fed 2.5 mg food/fish/day, (2) an overfed group fed 20 mg food/fish/day and (3) an overfed group fed 20 mg food/fish/day with 3 mg DEHP/kg food. Following a 60 day exposure, fish were euthanized, and the gut and fecal matter were excised. Fecal DNA was extracted and 16S conserved regions were sequenced, matched to known sequences from bacterial phyla, and analyzed using QIIME. RNA was extracted from the host gut and analyzed for differential gene expression using RNAseq. Preliminary results indicate that treatment with DEHP alters the β diversity of the gut microbiome. Specifically, this study noted an increase in Bacteriodetes phylum in the overfed with DEHP group alone, and decreases both Fusobacteria and Tenericutes phyla in both the overfed and overfed with DEHP groups. Such changes in the abundance of Bacteriodetes and Fusobacteria of the gut microbiome have previously been associated with obesity and colorectal cancer in humans. These data support the hypothesis that environmental exposure to phthalates may negatively impact fish species by altering the gut microbiome, which may subsequently alter the ability of the organism to process nutrients, leading to metabolic disruption.

WP152 Suitability of frog skin microbiomes as sentinels of endocrine disrupting compounds

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Municipal wastewater effluents introduce a variety of pharmaceuticals and personal care products (PPCPs) into the aquatic environment, which can perturb diverse biological processes by disrupting hormone function. Effective, environmentally-relevant methods to detect the activity of endocrine disrupting compounds (EDCs) are lacking, particularly for thyroid hormone (TH)-disruption. One model to test for EDCs is based on TH-dependent tadpole metamorphosis: tadpoles exposed to TH will become froglets, but this effect is blocked if TH-disrupting chemicals are present. Because this model relies on a binary outcome, it lacks sensitivity. Further, it looks at a single animal outcome instead of evaluating the impact of contamination on the whole community. While evaluating changes across a macrobiotic community is difficult, microbial communities provide an opportunity to profile population-level community changes. Bacterial communities can be taxonomically profiled by DNA-sequencing a phylogenetic marker gene (16S rRNA amplicon sequencing). Such profiling has already revealed that frog skin associated bacterial communities can reflect host changes. Therefore, we are investigating whether a microbiome-targeted assay would enhance existing tadpole-based tests for EDCs and TH-disruption. In order to investigate the effect of PPCPs on frog skin microbiomes, we are profiling their bacterial composition across chemical exposures and life stages. Skin microbiome samples were collected from Rana (Lithobates) catesbiana tadpoles exposed to TH, a cocktail of PPCPs, and suitable controls, all under conditions similar to those used for regulatory monitoring. Samples were also collected from unexposed animals across pre- and post-metamorphic life stages. Preliminary results suggest that frog skin bacterial communities vary by life stage and with exposure to TH. Experiments to evaluate the effect of PPCPs are currently underway. This study will provide fundamental baseline data for lab-reared frogs under controlled laboratory conditions, describing the variability of skin microbiomes over life stages and chemical exposures. The results of the present study will contribute to the evaluation of bacterial communities as sentinels of environmental contamination.

Strategies to Manage Contaminants in Urban Stormwater Runoff

WP153 Concentration and Forms of Carbon in a Longitudinal Gradient from Freshwater to Estuarine Ecosystem

G. Baysal, Univ of Florida IFAS; G.S. Toor, Univ of Florida / Soil Water Science Dept

Urbanization affects water resources and carbon (C) is one of the indicators to determine the effects of urbanization in coastal waters. We investigated the concentrations and forms of C in a longitudinal gradient from freshwater to estuarine ecosystem in Florida. Monthly water samples were collected (December 2015–May 2016) from 10 freshwater sites located in the upper (n=3), middle (n=4), and lower (n=3) reaches of a river and 3 estuarine sites. Water samples were analyzed for dissolved organic C (DOC), total organic C (TOC), and total C (TC). Other C forms were calculated as follows: particulate organic C (POC) = TOC–DOC; total inorganic C (TIC) = TC–TOC. Across freshwater and estuarine sites, TC was 10.0–47.8 mg/l, of which TOC was 8.2–65.1%, and TIC was 34.9–91.8 % during April–May 2016. Among organic C, DOC was the dominant form at all sites (>98%), whereas POC was < 2%. Concentrations of all C forms were lowest at the most upstream site, which continued to increase till middle reach of the river and then declined in the lower reach and estuarine system. The increase in C in the upper parts of the river is attributed to the increase in the urban land use. However, in the lower reach of the river, a part of the water is impounded in a reservoir to provide drinking water for local municipality. We suggest that water impoundment increased the residence time of water, which likely resulted in utilization of C as an energy source resulting in lower C concentrations. After water left the reservoir, another river added low C water resulting in dilution of C concentrations in the estuarine sites. The dominance of TIC across all sites is attributed to the geologic sources such as limestone in the watershed that likely contributed carbonates and bicarbonates. Among organic C, the dominance of DOC in this subtropical ecosystem is not surprising due to the abundance of organic sources such as plant materials and sediments. We conclude that concentrations and forms of of C were affected by urbanization, water impoundment, and dilution–where urbanization increased the concentrations in the freshwater system, whereas water impoundment and dilution decreased the concentrations in the estuarine system.

WP154 Evaluation of Turfgrass Variety and Management Practices to Mitigate Off-site Transport of Pesticides and Nutrients with Runoff from Golf Course Turf

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Managed turfgrass is found in both private and public settings; as residential, commercial and public lawns, on golf courses and athletic fields, along roadsides, and in parks and cemeteries. Turfgrass offers protection from soil erosion, provides habitat for wildlife, and offers ecosystem services including aesthetics promoting mental health and safe playing surfaces for children and athletes. Strategies used to maintain managed turfgrass often involve multiple applications of pesticides and fertilizer at rates that exceed application rates of cultivated farmland. Pesticides associated with the turfgrass industry have been detected in storm runoff and surface waters of urban watersheds; inferring contaminant contributions from residential, commercial, and recreational sources. Excess nutrients in surface waters result in enhanced algal blooms, promotion of eutrophication, and negative impacts on sensitive aquatic ecosystems. We evaluated the influence of turf variety and effectiveness of management practices to reduce runoff volume and quantity of pesticides and nutrients transported with runoff from turfgrass managed as a golf course fairway. The overall goal is to eliminate or minimize risk of adverse impacts to aquatic ecosystems receiving turfgrass runoff as well as improve efficacy of plant protection products at their site of application. Hydrographs and chemographs revealed both turfgrass variety and turf management practices influence runoff volume and off-site loads of applied chemicals. This information is useful to grounds superintendents when selecting best management practices and as data for watershed-scale modeling evaluating the effect of land management practices on surface water quality.

WP155 Nitrogen in Freshwater and Estuarine Ecosystems: Longitudinal Distribution, Source Characterization, and Bioavailability

J. Jani, Univ of Florida / Gulf Coast Research and Education Center; G.S. Toor, Univ of Florida / Soil Water Science Dept

Urbanization accelerates nitrogen (N) loss from land resulting in excess N in coastal waters, which leads to harmful algal blooms, hypoxia, and loss of essential habitat. Our objectives study were to (1) investigate longitudinal distribution of inorganic and organic N forms, (2) identify sources of nitrate (NO_3-N), and (2) determine dissolved organic nitrogen (DON) bioavailability in freshwater and estuarine ecosystems. Monthly water samples (December 2015–May 2016) were collected from 13 sites along a longitudinal gradient from freshwater (10 sites) to estuarine (3 sites) ecosystem. Samples were analyzed for various N forms [ammonium (NH_4), NO_3, DON, particulate organic nitrogen (PON)] and stable isotopes of N and O of NO_3. Bioassay experiments were conducted for 5 days to determine the bioavailability of DON. Mean concentrations of total N in freshwater sites were 0.5–1.2 mg/L and estuarine sites were 0.5–0.6 mg/L; these values were lower than EPA numeric criteria for freshwater (1.65 mg/L) and estuarine (1.125 mg/L) ecosystems.Organic N forms were dominant at all sampling sites (DON: 64%, PON: 22%) as compared to inorganic N forms (NO_3-N: 7%, NH_4-N: 7%). Stable isotope data showed that NO_3-N in urban waters originated from mixing of multiple sources and biotic processes (i.e. nitrification). The bioavailability of DON ranged from 13 to 65% across all sites, with highest bioavailability in the estuarine sites. We conclude that although the estuarine ecosystem had low inorganic N (< 15% TN), the higher bioavailability of DON will meet the N requirement of algae and bacteria, which may result in onset of algal blooms.

WP156 Sources and Transport of Nitrate-Nitrogen in Urban Residential Runoff

Y. Yang, Univ of Florida; G.S. Toor, Univ of Florida / Soil Water Science Dept

Urban development increases fluxes of water and pollutants in urban watersheds. We investigated the sources and transport of nitrate-nitrogen (NO_3-N) in urban residential stormwater runoff using N and oxygen stable isotope of nitrate ($\delta^{18}O-NO_3^-$ and $\delta^{15}N-NO_3^-$). Water isotopes ($\delta D-H_2O$ and $\delta_{18}O-H_2O$) measurements were used to determine the source of water in stormwater runoff. Stormwaer runoff were collected during 2014 wet season (June-Auguest) that received ~33% of ~130 cm annual rainfall from six medium- to high-density residential catchments located in Tampa Bay, Florida. Concentrations of NO_3-N in stormwater runoff varied from 0.07 to 0.29 mg/L, of which NO_3-N was < 40% and organic N was >60% across six residential catchments. Our results showed that atmospheric deposition (35-64%), followed by chemical N fertilizers (1-39%) were the dominant sources of NO_3-N in stormwater runoff. This study will discuss the source identified and importance of developing correct best management practices to reduce N export in urban water systems.

WP157 Longitudinal distribution of phosphorus fractions along an urban river

S. Asal, Univ of Florida IFAS / Gulf Coast Research and Education Center; G.S. Toor, Univ of Florida / Soil Water Science Dept

Phosphorus (P) is a limiting nutrient in freshwater ecosystems. Excess P in water bodies lead to eutrophication and can impact the economy. Our objective in this study was to determine the longitudinal distribution of various P forms in an urban river that drains to an estuary. Monthly water samples were collected along a longitudinal gradient (13 sampling sites) in three reaches of Braden River – upper, middle, and lower – and Manatee River in Florida from December 2015 to May 2016. Samples were separated into dissolved (≤0.45 µm) and particulate (≥0.45 µm) P forms using a phsicochemical fractionation method, which resulted in four P forms: dissolved reactive P (DRP), dissolved unreactive P (DUP), particulate reactive P (PRP), and particulate unreactive P (PUP). Among all P fractions, the most dominant P form at all sites over six months was DRP (mean: 99 ug/L; 46% of TP), followed by PRP (mean: 59 ug/L; 27% of TP), DUP (mean:39 ug/L; 19% of total P), and PRP (mean:15 ug/L; 8% of total P). However, the concentrations and proportions of P forms varied over time at different sampling sites. For example, the proportion of inorganic P forms were more variable (DRP: 25-57%; PRP: 5-59% of total P) as compared to organic P forms (DUP: 12-33%; PUP: 3-14% of total P). Total P concentrations at all sampling locations over six months did not exceed the US Environmental Protection Agency Numeric Nutrient Criteria thresholds (330−490 µg/L). Results suggest that understanding the sources of different P forms in water bodies is needed and will be a key to devising strategies to control P losses from land to water.

EDCs and Pharmaceuticals in the Environment

WP158 Quantification of Human Pharmaceutical Conjugates in Municipal Wastewaters

A. Brown, Univ of Manitoba / Chemistry; C.S. Wong, Univ of Winnipeg / Richardson College for the Environment

Recent data suggests there are non-trivial amounts of human pharmaceutical conjugates potentially entering environmental surface waters. These compounds could contribute to eliciting toxic effects either directly or indirectly (via de-conjugation) on aquatic biota. The need for developing a single method for quantifying both parents and conjugates is necessary. Propranolol (PRO), sulfamethoxazole (SMX), and their respective major conjugates 4-OH-propranolol sulfate (PRO-Sul) and sulfamethoxazole-β-glucuronide (SMX-Glc) were successfully simultaneously extracted through weak anion exchange solid phase extraction cartridges from primary and secondary clarification wastewaters from the North End

Winnipeg Water Pollution Treatment Plant in Winnipeg, Manitoba, Canada. Subsequent separation and quantification was achieved by reversed-phase C_{18} chromatography coupled to positive electrospray ionisation tandem mass spectrometry. Linearity was > 0.99, and recovery RSD ranges across all matrices for PRO, SMX, PRO-Sul, and SMX-Glc were 2.14-13.21%, 2.32- 10.18%, 9.79- 19.22%, and 2.01- 10.32% respectively. Primary and secondary filtrates respectively showed a significant increase of PRO from 0.039 to 0.045 µg/L (P= 0.0457); SMX showed a significant decrease from 1.56 to 0.58 µg/L (P< 0.0001); PRO-Sul showed a significant decrease of 0.050 to 0.020 µg/L (P= 0.0172); and SMX-Glc showed a significant decrease from 0.41 to 0.019 µg/L (P< 0.0001). To the best of our knowledge this is first study that simultaneously separated and quantified two different classes of parent compounds and two different kinds of human transformation product conjugates (glucuronide and sulfate) from a major urban wastewater treatment plant in Canada.

WP159 Pharmaceuticals and Personal Care Products in an Effluent-Dominated Stream: Seasonal Variability and Downstream Fate

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Wastewater treatment plants (WWTPs) are major sources of pharmaceuticals and personal care products (PPCPs) in the environment, particularly in effluent-dominated streams (EDSs). EDSs likely represent worst-case scenarios for exposure of PPCPs to aquatic organisms. Dilution often decreases concentrations of PPCPs in streams downstream from WWTP effluents but other attenuation mechanisms such as degradation, sorption to streambed sediments, and plant uptake may also be important for some PPCPs. However, data quantifying the impact of these mechanisms is generally lacking in most EDSs. Environmental conditions such as weather, temperature, precipitation and snowmelt can also impact seasonal PPCP concentrations in EDSs. The objective of this study was to investigate the attenuation and yearly concentration variability of PPCPs in the East Canyon Creek, an EDS in Park City, Utah. Not surprisingly, water samples collected from the East Canyon Creek show that PPCP concentrations below the East Canyon Water Reclamation Facility (ECWRF) discharge point are much higher than upstream concentrations. In addition, the variability of PPCP in the concentrations as a function of season, downstream location and physical-chemical properties of the PPCPs will be described.

WP160 The occurrence and fate of parabens and their metabolites in wastewater treatment plants in India

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Parabens (p-hydroxy benzoic acid esters) are antimicrobial agents, used widely as preservatives in personal care products (PCPs), pharmaceuticals, foods and beverages. Parabens are endocrine-disrupting chemicals (EDCs). The use of products that contain parabens results in continuous release of these EDCs into sewage treatment plants (STPs), which is a cause for concern. A few studies have demonstrated the occurrence of parabens in wastewater and indicated that conventional treatment plants are not adequate to remove parabens from water cycle. This is the first study to measure parabens in Indian wastewater and sewage samples. We focused on the occurrence and fate of six parabens, viz., MeP, EtP, PrP, BuP, HpP and BzP and their metabolites (4-HB, 3,4-DHB, OH-MeP, OH-EtP and BA) in influent, effluent and sludge samples collected from five sewage treatment plants and 6 raw sewage collected from sewerage channels in India. The sum of measured concentrations of parent parabens (∑parent parabens) were significantly lesser than the concentrations of their metabolites. The concentrations of parent parabens (∑parent paraben) in five STPs ranged between 16.8 and 222 ng/L in influent; 36 and 58.3 ng/L in effluent and 96.7 and 1090 ng/g, dry weight, in sludge samples. Similarly, the concentrations of paraben metabolites (∑paraben metabolites = 5) in five STPs ranged between 2650 and 32800 ng/L in influent; 1660 and 3690 ng/L in effluent and 1220-36000 ng/g, dry weight in sludge samples. We calculated the mass loadings, removal efficiency and the amount released into the environment based on the measured concentrations of parabens and their metabolites in influent, effluent and sludge samples.

WP161 Levels of PCBs and PBDEs in the liver of dolphins from the Brazilian southeastern coast

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Dolphins are very sensitive to environmental changes and have been considered a good bioindicator of contaminants. Persistent organic pollutants (POPs) are able to bioaccumulate in fat tissues and biomagnify through the food web. Some of them, like PCBs and PBDEs can act as endocrine disrupters (EDCs). Marine mammals are top predators and may accumulate high concentrations of organic pollutants through bioaccumulation and biomagnification processes. Despite the concern over the widespread distribution of PCBs and PBDEs few studies have reported their occurrence in South America. The objective of the present work was to determine the levels of PCBs and PBDEs in liver of two species of dolphins (Tursiops truncatus and Stenella frontalis) stranded and accidentally caught in the Brazilian southeastern coast in 2012. Only fresh carcasses were used in this study. About 1.0 g of sample was used in this work. The analytical procedure was performed based on an Ultra Turrax extraction followed by clean-up and chromatographic analysis (GC/MS). ? PCBs ranged from 3078 ng g^{-1} to 15306 ng g^{-1} (lipid wt.) in Tursiops truncatus and from 6091ng g^{-1} to 29138 (lipid wt.) in Stenella frontalis. ? PBDEs varied from 330.79 ng g^{-1} to 864.92 ng g^{-1} (lipid w) in Tursiops truncatus and from 397,20 to1685,81ng g^{-1} (lipid w) in Stenella frontalis. PCB 153, 138 and 180 were the major PCB congeners detected in both species. Furthermore, BDE 47 was the major PBDE congener found in both species. The contamination patterns suggest the previous use of Aroclor 1254, 1260 and Penta –BDE mixtures in Brazil. PCBs and PBDEs were never produced in Brazil. However, the concentration found in this study, are of great concern, suggesting the presence of a source contamination in the Brazilian southeastern coast and representing a risk to these cetacean species.

WP162 Human pharmaceuticals in fillet of fish species of interest for consumption in the Uruguay River

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The Uruguay River, is an important South American river that flows from north to south along 1,838 km and form part of the boundary of Brazil, Argentina, and Uruguay. CARU is a binational Commission created to administrate the shared sector of the river between Argentina and Uruguay and, among other responsibilities, it is aimed to protect the fisheries and living resources. Several cities are located along this sector of the river that receive its wastewater, raw or poorly treated. Human pharmaceuticals are excreted by the urine and feces through the sewage drain, and are unable to be fully degraded by treatment plants. Then, concern has raised on the potential accumulation of these compound on fish species that are usually consumed. In the present study the concentration of 20 pharmaceuticals were measured by ASE-GPC-HPLC-MS/MS in the muscle of three emblematic fish species: Prochilodus lineatus (sábalo), Leporinus obtusidens (boga) and Salminus brasiliensis (dorado) caught in 10 localities under the CARU jurisdictional area. Only 9, 6 and 3 of the 20 compounds were detected at least once in boga, sabalo and dorado, respectively. Carbamazepine was ubiquitous, but always below quantification levels. Atenolol, clopidogrel and metoprolol followed it in occurrence frequency. Maximum total concentration of the studied pharmaceuticals was found in boga (20.9 µg/Kg) and significantly higher values were observed for boga and dorado collected from the sites located in lower sector of the river compared with the intermediate and upper sectors. The highest concentrations were observed for propranolol (6.44 µg/Kg) and atenolol (5.49 µg/Kg) in boga. Although not specific regulation exist for this family of compounds in Argentina, the observed concentrations were below the maximum residues limits (MRL) established for pharmaceuticals by the European Union in food stuffs from animal origin (Commission regulation (EU) No 37/2010). This was the first report on bioaccumulation of pharmaceuticals of fish in the Uruguay River, and despite values are low and they should not pose a risk for human health, its presence would deserve more detailed studies and consider them in the monitoring programs.

WP163 Patterns and instream attenuation of select contaminants of emerging concern: Predicted and observed therapeutic hazards to fish respond to snowmelt

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Pharmaceuticals and other contaminants of emerging concern (CECs) are increasingly reported in the peer-reviewed literature. However, instream attenuation and seasonal dynamics of such observations are rarely studied, particularly for semiarid streams with flows influenced by seasonal snowmelt and municipal effluent discharges. Herein, fish plasma modeling appears to provide a useful approach to identify concentrations of pharmaceuticals in surface water that may increase risk to fish when internal fish plasma levels approach human therapeutic (Cmax). We selected East Canyon Creek, located in Park City, Utah, USA, because it experiences yearly snowmelt and thus has higher flows in spring, which dilutes reclaimed wastewater discharge, but experiences lower and increasingly effluent-influenced flows during summer and fall months. The primary objective of the current study was to evaluate longitudinal gradients of select pharmaceuticals and other CECs in surface waters upstream and at incremental distances downstream (0.15, 1.44, and 13 miles) from an effluent discharge to East Canyon Creek. This gradient was then sampled during spring, summer and fall 2014 to determine whether contaminant levels were differentially attenuated and responded to seasonally variable instream flows. A secondary objective was to examine potential risks of pharmaceutical exposure to brown trout (Salmo trutta). Isotope dilution LC/MSMS was used for the quantitation of target analytes in surface water, effluent and fish plasma samples. Preliminary data for the attenuation rate of target analytes suggests that caffeine, benzolecgonine (cocaine metabolite), and carbamazepine have the lowest attenuation rates and are recalcitrant. Further preliminary data suggests that amitriptyline, methylphenidate, and diphenhydramine had the highest attenuation rates. Amitriptyline, fluoxetine and norfluoxetine were generally predicted from water concentrations within an order of magnitude of the observed internal dose levels in S. trutta. Several antidepressants were observed to accumulate in plasma of S. trutta above a previously proposed safety factor. Levels of amitriptyline were specifically observed within one order of magnitude of its corresponding human Cmax in fish plasma during lower flows associated with the fall sampling event. Unfortunately, the bioaccumulation dynamics or toxicological implications of such observations are not understood but warrant additional study.

WP165 Multiclass Endocrine Disrupting and Pharmaceutical Chemicals in Sediments and Surface Water near a Potomac River Wastewater Treatment Facility

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As awareness of the multiple classes of endocrine disrupting (EDCs) and pharmaceutical chemicals found in the environment grows, wastewater treatment plant operators look to determine what impact if any that their effluent has on the aquatic ecosystems and drinking water sources in the vicinity. This begins with the determination of fate and transport data. Water, sediments, and suspended-sediment samples were collected from the Potomac River in Alexandria, Virginia as well as from Hunting Creek, a small tidal tributary into which treated municipal wastewater is discharged. Water and suspended sediment samples were collected in 20 L stainless steel kegs. The water was pressure filtered with 145 mm diameter 0.2 um clean glass-fiber filters held in a stainless steel filter stand using high-purity nitrogen. The filtrate water was aliquoted into 1 L amber glass bottles and was extracted using mixed-mode solid phase extraction cartridges for EDCs or with a combination strong-anion and strong-cation exchange SPE method for pharmaceutical compounds. Sediment samples were ground and desiccated with anhydrous sodium sulfate and solvent extracted by a microwave assisted reaction system (MARS). Suspended sediment containing glass fiber filters were extracted by MARS as well. Sediment and filter extracts were cleaned either by florisil columns for EDCs or by SPE following dilution in UHP water for pharmaceuticals. Samples were analyzed by GC-MS or by LC-MS for estrogens, corticosteroids, sunscreen agents, antibiotics, over-the-counter pharmaceuticals, antidepressants, and household products. Notable detected water concentration ranges (ng/L) were bisphenol A 33 – 1632, prednisone 335 – 1255, diclofenac 126 – 372, sulfamethoxazole 26 – 206, naproxen 82 – 101 and caffeine 35 – 70. Mean concentrations (ng/L) for Hunting Creek versus the Potomac River were higher with 575/205, 1469/208, 463/83, 300/11, for bisphenol A, prednisone, diclofenac, sulfamethoxazole, naproxen and caffeine respectively. The experimental K_{oc} partition coefficient values for sediments and suspended sediments generally gave mixed results when compared with their theoretical values.

WP166 Transport of Steroidal Hormones from Cattle Feedlot Pens via Simulated Rainfall Runoff

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Growth promoters including steroidal hormones such as trenbolone acetate and melengestrol acetate are routinely administered to cattle in commercial feedlots to improve feed efficiency and promote growth. Trenbolone acetate (TBA) is a synthetic androgenic steroid used as an anabolic growth promoter administered via implants while melengestrol acetate (MGA) is a synthetic progestogen administered orally to heifers to improve feed conversion, promote growth, and suppress estrus. TBA, MGA and their metabolites are excreted by cattle in feces and urine. These contaminants could be introduced into the environment when the corresponding manure is applied as fertilizer to agricultural fields or through leaching or runoff from manure storage locations (feedlots, stockpiles, windrows, lagoons). These steroids and their metabolites have been detected in manure, feedlot runoff, catch basin water, and manure-amended soils. Runoff after major precipitation events appear to be major transport pathways by which these steroidal hormones from manure eventually contaminate surface water. A study is being conducted at the Agriculture and Agri-Food Canada Research Centre, Lethbridge, AB to investigate the transport in simulated rainfall runoff of TBA and MGA from feedlot pens. Cattle were administered the following steroidal hormone treatments according to Compendium of Veterinary Products: (1)

0.4 mg of MGA head^{-1} d^{-1} via feed, (2) 200 mg of TBA head^{-1} for every 84 d via implants, (3) no MGA or TBA (control). After manure accumulation in the pens, cattle were relocated and rainfall simulation experiments were conducted in situ using a portable Guelph Rainfall Simulator II. Composite manure samples were collected from the pen floor prior to rain simulations. Manure and runoff samples will be extracted and the extracts analyzed for MGA and TBA using LC/MS/MS analysis. The residue data will be used to determine the amount of each steroidal hormone that would potentially be transported to the catch basin adjacent to the feedlot in the event of a major rainfall.

WP167 Fate and Transport of Bisphenol A, F and S in Soil Irrigated with Wastewater

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There is a growing concern about the occurrence of bisphenols in the environment. They are widely used in the synthesis of polycarbonate plastics and epoxy resins. Therefore, they are commonly found in consumer goods and containers. Bisphenols have the ability to disrupt the endocrine system and cause harmful effects. The potential uptake and distribution of bisphenol A and bisphenol F in edible crops and their transport in soil needs to be closely examined. Therefore, the focus of this study is to (1) investigate the fate and transport of bisphenol A, F and S in potato tubers in soil irrigated with untreated wastewater; (2) Examine the effect of three amendments, plantain peel biochar, super absorbent polymer (SAP) and the combination of biochar and SAP, in reducing plant uptake and pollution. A four-month study in a field lysimeters, grown with potatoes, was conducted to investigate the abovementioned goals. Results showed that none of the tested bisphenols was found in the flesh of potato tubers after harvest. However, the concentration of bisphenols in topsoil increased over the growing season, indicating the cumulative effect in the soil. The biochar treatment indicated higher removal efficiency (45%) of bisphenols compared to other treatments.

WP168 Reproductive and general health assessment of fathead minnow populations inhabiting an effluent-dominated stream, Wascana Creek, SK, Canada

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The province of Saskatchewan is experiencing a dramatic increase in population growth, resulting in a greater release of municipal wastewater effluents (MWWEs) into local water bodies. There is concern regarding the impact of contaminants in this effluent, particularly endocrine disrupting compounds (EDCs), to resident wildlife as conventional wastewater treatment technologies are often incomplete or inefficient at removing such compounds. Waterbodies in the southern Canadian Prairies may be at particular risk of exposure to EDCs due to the uniqueness of prairie surface water systems. For example, during low flow periods, Wascana Creek, a small stream in southern Saskatchewan can consist of over 90% treated effluent originating from the City of Regina's outdated lagoon based treatment facility. The aim of this study was to characterize the potential endocrine disrupting effects of municipal waste-water effluents on wild fathead minnow (Pimephales promelas; FHM) populations in an effluent dominated stream, Wascana Creek, SK. Field studies were conducted on spawning FHMs (2014 and 2015) to assess responses in terms of overall health (condition factor, somatic indices), reproduction (secondary sexual characteristics, sex steroids in blood plasma, gonad histopathology, gene expression), and sex ratios. Fish collected downstream of the effluent fallout had lower gonadosomatic indices and significantly greater hepatosomatic indices compared to fish from upstream populations. There was significant disruption of regulation of key genes along the hypothalamus-pituitary-gonad-liver axis that are associated with reproductive processes. Additionally, in both male and female FHMs gonadal degradation and delayed maturation was observed histologically. Exposed males displayed lower scores of secondary sexual characteristics. This case study highlights the potential ecological risks of EDCs associated with MWWEs, and the need for implementing more effective and affordable measures to remove them at wastewater treatment plants.

WP169 Degradation Pattern of pharmaceutical and personal care products (PPCPs) mixture and its effect on Soil Microbial diversity

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Pharmaceutical and personal care products (PPCPs) associated with land farming of municipal wastewater effluent may potentially, persist in the soil and alter soil microbial community processes. Estrone (E1) and the anti-microbial agent, Triclosan, were examined for their potential to persist and disrupt soil microbial community function. Soil with 7 decades-long exposure to these chemicals (conditioned soil) and naive soil, which has not been previously exposed (unconditioned soil), was spiked with estrone, triclosan, or a 1:1 mixture of estrone: triclosan, and incubated for 90 days in the dark at 27°C. Control samples consisting of unspiked conditioned and unconditioned soil were included in the analysis. The community level physiological profile was examined using BIOLOG® EcoPlates™ for the ability of their microflora to utilize ecologically relevant carbon sources. There was a significant increase in substrate activity and substrate richness in all treatments. Principal component analysis (PCA) of the data showed the microbial community utilized different carbon substrates by day 90 whereas they had exhibited similar substrate utilization at day 0. Microbial degradation rates were compared over the 90 days incubation period using high performance liquid chromatography (HPLC). Estrone and Triclosan showed the same pattern of biological degradation in both conditioned and unconditioned soils. Half-lives were determined to range between 5.9-6.8 days for the estrone treatments and 24.1-26.9 days in the triclosan treatments. The rate of degradation of the estrone:triclosan mixture was the same as the individual compound.

WP171 Microsomal metabolism of trenbolone acetate metabolites: Transformation product formation and bioactivity

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Trenbolone acetate (TBA) is a synthetic growth promoter widely used in animal agriculture, and its metabolites are suspected endocrine disrupting compounds in agriculturally impacted receiving waters. However, beyond the three widely recognized TBA metabolites (17b-trenbolone, 17a-trenbolone and trendione), little is known about other metabolites formed in vivo and subsequently discharged into the environment, with some evidence suggesting these unknown metabolites may comprise a majority of the TBA mass administered to the animal. Here, we explored the metabolism of the three known TBA metabolites using rat liver microsome studies. All TBA metabolites are transformed into a complex mixture of monohydroxylated products. Based on transformation product characterization, the majority are more polar than the parent metabolites but maintain their characteristic trienone backbone. A minor degree of interconversion between known metabolites was also observed, as were higher order hydroxylated products with a greater extent of reaction. Notably, the distribution and yield of products were generally comparable across a series of variably induced rat liver microsomes, as well as during additional studies with human and bovine liver microsomes. Bioassays conducted with mixtures of these transformation products suggest that androgen receptor (AR) binding activity is diminished as a result of the microsomal treatment, suggesting that the transformation products are generally less potent than the parent TBA metabolites from which they were generated. Outcomes of this work are beneficial in attempting to more confidently assess the environmental occurrence and ecosystem risks associated with TBA use in animal agriculture.

WP172 Triclosan is a proton ionophore mitochondrial uncoupler super-resolution microscopy reveals disrupted mitochondrial ultrastructure

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Triclosan (TCS) is an antimicrobial used so ubiquitously that seventy-five percent of the U.S. population is likely exposed to TCS via consumer goods and personal care products that contain concentrations up to 10mM TCS. TCS is readily absorbed into human skin and has been found in urine and in the plasma and milk from nursing mothers. Detectable levels of TCS have also been found in a wide range of aquatic and terrestrial wildlife. We found that non-cytotoxic, µM levels of TCS inhibit both adenosine triphosphate (ATP) and oxygen consumption rate (OCR), from rat (RBL-2H3) and human (HMC-1.2) mast cells, NIH-3T3 mouse fibroblasts, and primary human keratinocytes. Primary human keratinocytes yield the most pronounced decrease in ATP with an EC_{50} of 3.0-4.1 µM (95% CI). This effect is due to TCS's proton ionophore structure. The reduction in ATP with no change in plasma membrane integrity in multiple cell types, including primary human cells, indicates that TCS is a mitochondrial toxicant. Known mitochondrial uncouplers have been shown to disrupt mitochondrial morphology. We have successfully imaged mitochondrial ultrastructure using the super-resolution microscopy technique fluorescence photoactivation localization microscopy (FPALM) with the outer mitochondrial membrane marker dendra2-TOM20 and have shown that TCS changes mitochondrial morphology without causing apoptosis. TCS also decreases mitochondrial membrane potential. TCS increases reactive oxygen species (ROS) production in RBL-2H3 cells, in contrast to the effects of the known uncoupler CCCP, which decreases ROS production. These data show that TCS disrupts mitochondrial and cellular functioning in diverse cell types.

WP173 Triclosan is a mitochondrial uncoupler in live zebrafish

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Triclosan (TCS) is a synthetic antimicrobial agent used in many consumer goods at millimolar concentrations. As a result of exposure, TCS has been detected widely in humans. We have recently discovered that TCS is a proton ionophore mitochondrial uncoupler in multiple types of living cells. Here we present novel data indicating that TCS is also a mitochondrial uncoupler in a living organism: 24 hour post fertilization zebrafish embryos. These experiments were conducted using a Seahorse Bioscience XF^e 96 Extracellular Flux Analyzer modified for bidirectional temperature control, using the XF96 spheroid plate to position and measure one zebrafish embryo per well. Using this method, following acute exposure to TCS, basal oxygen consumption rate (OCR) increases, without a decrease in survival or heart beat rate. TCS also decreases ATP-linked respiration and spare respiratory capacity and increases proton leak: all indicators of mitochondrial uncoupling. Our data indicate, for the first time, that TCS is a mitochondrial uncoupler in vivo, which should be taken into consideration when assessing the potential toxicity and/or pharmaceutical uses of TCS. To our knowledge, this is the first example of the Seahorse 96-well Extracellular Flux Analyzer being used to measure bioenergetic flux of a single embryo per well in a 96 well assay format. The method developed in this study provides a novel high-throughput tool to identify previously-unknown mitochondrial uncouplers in a living organism.

WP174 Interactions among diverse endocrine disrupting chemicals: Tetrabromobisphenol A modulates estradiol and bisphenol A concentrations in mice

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Many toxicological studies examine the effects of a single chemical, whereas people are commonly exposed to multiple toxicants that may interact. Here we studied interactions of a flame retardant, tetrabromobisphenol A (TBBPA), with two other environmental chemicals: bisphenol A (BPA; the monomer of polycarbonate plastics) and triclosan (an antimicrobial agent). These chemicals are found in numerous consumer products and are absorbed into the body through the skin, lungs, and gastrointestinal tract. Whereas only BPA shows affinity for estrogen receptors, all three chemicals interact with key metabolic enzymes, including sulfotransferase and UDP-glucuronosyltransferase. We hypothesized that TBBPA may compete with estradiol and BPA for access to these enzymes, thereby elevating estradiol and BPA concentrations. We subcutaneously injected mice with TBBA, administered 50 ug/kg 14C-BPA in a dietary supplement, and subsequently measured levels of radioactivity in serum and tissues. A single injection of TBBPA magnified ^{14}C-BPA concentrations in serum and reproductive organs in a dose-dependent manner. Moreover, concurrent treatment with TBBPA and triclosan resulted in higher concentrations of ^{14}C-BPA than either chemical given alone. Administration of TBBPA also elevated urinary concentrations of endogenous estradiol in female mice. These data are consistent with a proposed adverse outcome pathway in which the molecular initiating event for TBBPA is the binding and inhibition of sulfotransferase. These data also provide convincing evidence of an in vivo interaction between environmental chemicals and steroid hormones. These findings demonstrate the importance of considering studies of multiple toxicants when determining regulatory exposure limits.

WP175 Characterization of the contribution of a major hospital in Central America to the load of pharmaceutical to urban wastewaters and receiving waters

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Many studies around the world have shown the presence of pharmaceuticals as emerging organic contaminants in surface waters and wastewaters. However, there is very limited knowledge about the occurrence and hence effects of these chemicals in the Central American tropical aquatic environment. In this study, passive sampling was used to determine time-weighted averages and fluxes of a suite of pharmaceuticals in the effluent of a major urban hospital in the metropolitan area of San Jose, Costa Rica. Concentrations and fluxes were also measured at the intake of San Jose's wastewater treatment plant, to ascertain the contribution of hospitals to the amount of drugs that could be attributed to releases from hospital compared to use by the urban population served by the treatment facility. Use and prescription records of the hospital were correlated with levels observed in hospital effluent, suggesting that these data could be used to predict the contribution of hospitals to pharmaceutical contamination in urban wastewaters. Key words: Pharmaceuticals, emerging contaminants, hospital, Costa Rica

WP176 The estrogen potency and reproductive impairment of equine estrogens on Japanese medaka (Oryzias latipes)

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The objective of this study was to investigate endocrine-disrupting potentials of equine estrogens (EQs) on Japanese medaka (Oryzias latipes). Our gene expression analysis with DNA microarray representing 6000 medaka genes revealed that 24-h exposure of 100 ng/L equilin (Eq) up-regulated the expression of 6 genes in male liver, and 24-h exposure of 100 ng/L equilenin (Eqn) also up-regulated the expression of 34 genes. The genes up-regulated by Eq included estrogen-responsive genes, such as vitellogenins (vtgs) and choriogenins (chgs), and among the up-regulated genes by Eqn exposure were cancer-related genes, such as mediator complex subunit 16 and RAS oncogene family members. We further investigated the gene expressions of vtgs, chgs, and estrogen receptor (ER) subtypes in male liver by qRT-PCR after exposing medaka to six different EQs (1-300 ng/L each) for 3 days. As a result, the estrogenic potentials of the chemicals were in the order of Eq > 17β-estradiol (17β-E_2) > Eqn > 17β-dihydroequilin > 17β-dihydroequilenin > 17α-dihydroequilin > 17α-dihydroequilenin, which showed the higher estrogenic potential of Eq than that of 17β-E_2. We were also interested in reproductive impairment in paired medaka that were exposed to 10, 100, and 1000 ng/L of Eq for 21 days, and an observation was noted that Eq (100 and 1000 ng/L) adversely affected the reproduction (fecundity and fertility) of adult medaka and hatchability in F1 generation fertilized eggs.

WP177 Effects of full-scale ozonation of treated effluent – Environmental impact in a receiving river

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One class of new emerging contaminants that have received a lot of attention lately are the pharmaceuticals. Pharmaceuticals are a diverse group of chemicals used in large quantities and are, in contrast to most chemicals used in society, designed to cause a specific pharmacological effect in biological systems. Pharmaceuticals have been found in aquatic systems globally, due to a combination of worldwide usage and low removal efficiency in wastewater treatment plants (WWTPs), or a complete lack of WWTPs (1). In surface waters, concentrations of pharmaceuticals usually range from low $\mu g\ l^{-1}$ close to point sources to low $ng\ l^{-1}$, and are correlated to human population density in the drainage area, volume of the receiving water body and technologies used in WWTPs. One technique to increase the removal of pharmaceuticals in WWTPs is to add a tertiary treatment step based on the addition of ozone. Ozonation is a cost efficient way to degrade chemicals and several studies have shown that most pharmaceuticals are readily degraded in the presence of ozone (2). However, several oxidized degradation products are formed during ozonation and the environmental impact of these are largely unknown. The aim with this study was to evaluate the removal of pharmaceuticals in a WWTP, when adding ozonation as an additional tertiary treatment step and also to investigate the environmental impact of this effluent on the receiving river. All treated effluent from a minor WWTP (10000 PE) were treated by an addition of 8 mg h^{-1} ozone during 6 months. Removal rates in the WWTP as well as levels of pharmaceuticals in the receiving river (both in water and biota) were monitored. Ecological status was also determined before, during and after the time period when the ozonation treatment step was used. Several methods were used including invertebrate inventories, microbial community composition, etc. 1) Lindberg RH, Östman M, Olofsson U, Grabic R, Fick J. 2014. Occurrence and behaviour of 105 active pharmaceutical ingredients in sewage waters of a municipal sewer collection system. Water Research58, 221-229. 2) Ikehata K, Jodeiri, N, Naghashkar MG.El-Din. 2006 Degradation of Aqueous Pharmaceuticals by Ozonation and Advanced Oxidation Processes: A Review, Ozone: Science & Engineering, 28:6, 353-414

WP178 A meta-analysis of parabens in sewage sludge

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Parabens are a group of compounds extensively used as preservatives in pharmaceutical and personal care products (PPCPs), food, beverages, and industrial products. They also are considered to represent emerging endocrine disruptors and have raised concerns for human and animal health. Parabens are known to be toxic to aquatic organisms and also potentially may cause immune dysfunctions and adverse reproductive outcomes in humans. Widespread use of parabens can lead to steady-state levels in the environment, with wastewater treatment plants (WWTPs) and sludge disposal representing two important sources. Despite their biodegradability, parabens remain detectable at ng/L levels in treated plant effluent, and at ng/g dry weight levels in digested sewage sludge. Whereas parabens dissolved in WWTP process streams have been studied extensively, few studies have explored their occurrence and concentration in sewage sludge. Information on parabens in sludge is needed for risk assessments, since sewage sludge application on land represents a common disposal practice, potentially leading to contamination of soil, groundwater and surface waters. In this study, we reviewed literature data on parabens in sewage sludge, and estimated the estrogenicity contributed by parabens in sludge using as an indicator the Estrogenic Equivalent Quotient (EEQ). Parabens were found to be ubiquitous in sewage sludge, with methyl paraben and propyl paraben being the most frequently used and detected representatives of this chemical group. Overall, concentrations for parabens in sludge were moderate and varied between countries (n = 5) and sludge types. The overall theoretical estrogenicity burden contributed by parabens in sludge was less than 1 pg/g of 17β-estradiol equivalents or less than 0.1% of the burden posed by natural estrogens from food items present in sludge. This study indicates that the risk associated with synthetic parabens in sewage sludge is minor and essentially insignificant due to the comparatively low concentrations and estrogenicity. Future studies should include metabolites and derivatives of parabens, which also may act as potential endocrine disruptors and can occur in sewage sludge at concentrations higher than those of the parent compounds.

WP179 Oxidative stress-induced toxicity in Clarias gariepinus (Burchell, 1822) exposed to sub-lethal concentrations of pharmaceutical effluent

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Contamination of aquatic environment with industrial effluent has become a matter of great concern because of its toxicity and persistence inducing alterations in the chemical composition of aquatic environment. This, in turn, alters the behaviour and biochemistry of the aquatic fauna among which is catfish, Clarias gariepinus. In this study, pharmaceutical effluent sourced from the discharge point of a pharmaceutical industry in Kwara State was analyzed to determine its physicochemical parameters, the sub-chronic toxicity and biochemistry of alterations in tissues and organs of post juvenile Clarias gariepinus. The impact of long term exposure to the effluent was evaluated through growth performance and selected anti-oxidant enzyme activities using 0, 2, 4, 6, 8 and 10 % concentrations of the effluent for 21 days, following a range finding test. Also, the physicochemical parameter of the culture water were determined. The results of the physicochemical analysis of the effluent showed that heavy metal concentrations were below the national (FEPA and NESREA) and international (USEPA) limits for aquaculture while other parameters like dissolved oxygen, alkalinity and total hardness deviated from the standard. C. gariepinus exposed to sub-lethal concentrations of effluent showed increased growth performance and nutrient utilization at the 10% effluent concentration. Biochemical studies carried out on the gill, fin, liver and muscle showed that activities of anti-oxidative stress enzymes, Superoxide Dismutase (SOD), Catalase (CAT), and Glutathione-STransferase (GST), were higher in the liver of the effluent exposed C. gariepinus when compared to other tissues. In this study, the effluent had pronounced effect on the growth and enzymatic activities of studied tissues and organs of C. gariepinus. Conclusively, this study revealed that the pharmaceutical effluent, although treated is still a potent contaminant to post juvenile C. gariepinus.

WP180 CECs in the Wastewater and Biosolids of Calgary, Alberta: Fate, Trends and Environmental Impacts

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Municipalities are faced with an increasing number of questions from the public as to the presence and potential impacts of contaminants of concern (CECs) in wastewater effluent and biosolids. In order to develop an understanding of the occurrence, fate and potential impacts of CECs in Calgary's wastewater and biosolids, The City initiated monitoring programs in 2007 and 2011, respectively. The CECs investigated included: pharmaceuticals and personal care products, surfactants, hormones, plasticizers, perfluorinated compounds, synthetic musk fragrances, and flame retardants. Initial efforts were focussed on characterizing the final effluent and treated biosolids destined for land application, but the program was later expanded to include several other points in the treatment process. Calgary has 3 wastewater treatment plants (WWTPs), one with a distinctly different treatment process than the other two. Comparison of the influent and effluent concentrations between WWTPs suggests that the plants with biological nutrient removal were more effective at reducing certain CECs than the one with chemical nutrient removal. Separating the liquid and solid fractions of the sludge prior to analysis allowed for assessment of partitioning behaviour and for calculation of partition coefficients. Analyses of whole samples of digested sludges and lagoon-settled biosolids indicated that there is an overall increase in concentration for some CECs subsequent to lagoon-settling, particularly for the more hydrophobic contaminants. Although there are currently no regulations for CECs in wastewater or biosolids in Canada, guidelines are beginning to emerge at the federal and provincial levels for surface water for the protection of aquatic life. Concentrations of CECs in Calgary's downstream receiving waters will be compared to current guidelines to assess potential ecological effects.

WP181 Sucralose: a wastewater tracer or a water quality indicator?

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Traditionally, water quality investigations are geared to measure the occurrence of common chemical contaminants such as nutrients, priority pollutants and or bacterial indicators that are linked to well known detrimental effects on the receiving water bodies. Eutrophication, is easily recognized since the increased introduction of nitrogen and phosphorous to a body of water results in a cascade of visible signs such as algal blooms followed by hypoxia that may result in lethal effects for fish or other organisms. The effects are well known but the sources of the contamination are not always easily identifiable. Nutrients have a number of potential sources such as agricultural runoff, storm water runoff, domestic wastewater disposal or even septic tanks. There is no easy way to fingerprint the provenance of inorganic nutrient species. Many chemical tracers that are unique to human consumption have been proposed in the past. Among them, caffeine and now sucralose have been proposed a "tracers" to follow the intrusion of human derived wastewater into aquatic ecosystems. Nevertheless, for a tracer to become an indicator a relationship to traditional water quality parameters must be identified so a potential link to common impacts such as eutrophication can be elucidated. Such relationship between caffeine and elevated human-derived pathogens has been previously established but the concentration at which the relationship is evident (>400 ng/L) is too high to have environmental relevance. Sucralose, on the other hand has been explored as a tracer but not as an indicator. The purpose of this work was to add routine measurements of sucralose to a comprehensive water quality monitoring effort in a system of canals in the Florida Keys that have been subject to a conversion from traditional septic systems to municipal sewage collection. Statistical analysis of time series of traditional water quality parameters and sucralose showed an upward change in the trends of nutrients and dissolved oxygen for concentrations of sucralose above 57 ng/L. Cluster analysis indicated that samples whit sucralose levels below the threshold did not show evidence of water quality issues while concentrations above 53 ng/L and above 150 ng/L grouped the sites influenced and affected by eutrophication respectively. This study presents the first application of sucralose as an effective human-derived wastewater "indicator" rather than just a tracer.

WP182 Assessing Poultry Litter Composting Techniques and their Impact on Estrogen and Pharmaceutical Concentrations

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Confined animal feeding operations (CAFOs) of poultry generate significant amounts of litter which is often used to fertilize farmlands. The raw (untreated) poultry litter is known to contain naturally excreted estrogens as well as pharmaceuticals that were given to the chickens. These compounds can contaminate surface waters through runoff. Poultry litter can be applied in the composted, pelletized, or raw form. Composting poultry litter is advantageous because it decreases noxious odors and toxins and lowers the rate of nitrogen mineralization, which lowers the potential risk of contaminating the groundwater with high nitrate levels. This process is carried out using forced aeration and mechanical turning techniques to accelerate the degradation process by controlling moisture, temperature, and oxygen levels. In order to determine if different composting methods are effective at reducing estrogen and pharmaceutical levels in poultry litter a variety of pilot-scale (two ton capacity) composters were utilized. The poultry litter was obtained during a whole-house cleanout of a conventional broiler operation. The poultry litter was divided into four treatment categories: aerated, turned, turned and aerated, and piled (not composted). At the end of composting, samples were collected and lyophilized. Pre and post composting samples were extracted using solid-liquid extraction, followed by concentration and further cleanup using solid phase extraction, before analysis by liquid chromatography tandem mass spectrometry. Analytes of interest included macrolides, sulfonamides, tetracyclines, and both natural and conjugated estrogens; however, no sulfonamides or tetracyclines were detected in the samples. Tylosin, erythromycin and clarithromycin (which are all veterinary macrolide antibiotics) were detected in addition to the estrogens. Of the compounds detected, tylosin had the highest removal after all composting techniques while the removal of the other macrolides and estrogens favored certain composting treatments over others.

WP183 Factors controlling antibiotics levels in biosolids

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Removal rates of pharmaceuticals and personal care products during wastewater treatment are of great interest. The Targeted National Sewage Sludge Survey administered by the USEPA collected samples of biosolids from wastewater treatment plants (WWTP) across the US using a variety of treatment trains. Factor analysis was applied to antibiotic concentrations. Then comparisons of WWTP influent levels of antibiotics from a broad literature review were made to the antibiotics observed in biosolids. Treatment processes, biosolids class, and other key treatment factors were examined as possible explainers for the differences observed between WWTP influent levels from the literature and levels of antibiotics in biosolids. This data mining effort provides insight into the fate of antibiotics during wastewater treatment.

WP184 Exposure to municipal wastewater effluent influences fecundity and and hormone-signaling pathways in fathead minnows (Pimphales promelas)

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Municipal wastewater effluents (MWWEs) contain anthropogenic substances that can exhibit endocrine disrupting activity. In the Prairie provinces of Canada, increasing urban populations and industrial activities has meant greater water demand and therefore greater MWWE released into the aquatic environment. The objectives of this study were to (1) determine the biological effects of MWWEs on fathead minnow (Pimephales promelas) using the USEPA short-term fish reproductive bioassay assay and (2) compare responses to effluents collected from both the Regina and Saskatoon wastewater treatment systems. Fish were exposed for 21 days to 0 %, 10% and 50% effluent. Impacts on reproductive success (fecundity, fertility) as well as morphological, histopathological, and molecular/biochemical indicators were assessed in male and female fish. Exposure to Regina MWWE resulted in a significant decrease in fecundity compared to non-exposed minnows. Fecundity was increased in fathead minnow exposed to 50% Saskatoon effluent treatment when compared to the control. Fertilization rate remained consistent regardless of treatment. Histological examination revealed an increase in proportion of spermatogonia and testicular degeneration in male fathead minnow testes in 50% effluent concentrations compared to control minnows in both Regina and Saskatoon exposures. There was no change in female histopathological criteria with effluent exposure and to effects of either effluent on circulating sex steroid hormones (estradiol, 11-ketotestosterone) in male or female minnows. Transcriptional analysis was conducted for various genes involved in hormone signaling and steroidogenesis. There was no induction of vitellogenin in male or female fathead minnows exposed to Regina or Saskatoon MWWE suggesting a lack of estrogenicity. Induction was observed in select target genes but did not demonstrate a clear mechanism of endocrine disruption by either effluent source. This study determined functional reproductive impacts of short-term exposure to MWWE discharged into Saskatchewan aquatic systems but mechanism of action and causative agents remain to be identified.

WP185 Larval Zebrafish (Danio rerio) behavioral screen identifies altered spontaneous behavior with neuroactive pharmaceutical exposure

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Neuroactive pharmaceuticals (NP) are a diverse group of drugs designed to target the brain (e.g. antidepressants, anticonvulsants, antipsychotics, etc.) and are increasingly detected in the aquatic environment as human use of NPs increases. NPs have been detected in surface water and fish tissue samples around the world at concentrations ranging from ng to ug/L levels, thus the potential impact NPs have on aquatic ecosystems needs to be evaluated. Conserved structure of both the nervous systems and genomes of fish and mammals suggest that NP exposure is very likely to have behavioral effects on fish as well. Due to the rapid development and use of NPs, a sensitive high throughput procedure is necessary to characterize toxicological impacts of NP exposure. We've developed a behavioral screen using larval zebrafish (Danio rerio) that tests a wide variety of fish behaviors, ranging from spontaneous behaviors to simple cognitive processes. Preliminary work with the model antidepressant fluoxetine indicates decreased spontaneous locomotor behavior and startle response. Evaluation of many other NPs, including haloperidol, citalopram, lamotrigine, amitriptyline, primidone, bupropion, risperidone, paroxetine, sertraline, venlafaxine, oxazepam, carbamazepine and their metabolites, as well as waste water treatment effluent are currently underway. This protocol allows us to screen and compare a large number of compounds, mixtures, or environmental samples quickly, allowing us to rank their potency as modulators of early life stage fish behavior and inform more detailed work on modes of action.

WP186 The removal of endocrine disrupters using TiO2 immobilized on porous titanium sheets

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The insufficient removal of endocrine disrupters from wastewater discharged into nearby watercourses can pose risk to aquatic and human health. Most conventional treatment systems were not designed to remove these compounds and additional treatment options must be explored. The use of TiO_2 photocatalysis, a potentially low-cost advanced oxidation process, has recently been the subject of several water treatment studies. In order to increase its feasibility in water treatment applications, TiO_2 has been modified as nanomaterials or immobilized on a variety of supports such as porous alumina and polymeric membranes. In this study, immobilization was completed by thermo-chemically oxidizing the porous titanium supports, producing a self-assembly of TiO_2 on the surface. The efficiency of the immobilized TiO_2 (UV-LED irradiated) to degrade a mixture of five estrogenic compounds (17α-ethinylestradiol, 17β-estradiol estrone, estriol, and bisphenol A) at different pH conditions was assessed. The compound disappearance was determined using LC-MS/MS and a yeast-estrogen screen assay was employed to assess the ability of the synthesized material to remove the biological activity associated with these compounds. Removals were observed for all compounds except 17β-estradiol and lower pH conditions showed better removal. Although 17β-estradiol was found to be poorly degraded by the immobilized TiO_2, the total estrogenic activity decreased over time for all pH conditions. Since the rest of the compounds (17α-ethinylestradiol, estrone, estriol, and bisphenol A) expressed 60% of the biological activity, their simultaneous removal substantially reduced the total estrogenic activity even with the inefficient degradation observed for 17β-estradiol. Overall, the results show that the combination of UV-LEDs and the newly synthesized TiO_2 material is capable of significantly removing selected target estrogens and their associated biological activity.

Contaminated Sediment Remediation and Restoration: Assessing and Measuring Effectiveness

WP187 Challenges of Reliably Assessing Contaminant Exposure in Migratory Waterfowl at Contaminated Sites

J. Collins, B.J. Reese, AECOM; R.G. Stahl, E.I. DuPont de Nemours and Company / Corp Remediation

Mercury concentrations in mallard duck (Anas platyrhynchos) tissue are being monitored as part of the Long-term Monitoring (LTM) program for a mercury impacted riverine system in the Shenandoah Valley, Virginia. The primary objective of the monitoring program is to evaluate potential changes in human exposure to site-related mercury contamination through the consumption of mallard ducks harvested within the system in response to remediation. The project study area spans a contamination gradient over 160 river miles and includes aquatic habitats influenced by a diverse spectrum of land uses including, urban, agricultural and national forests. Reliably assessing mercury exposure at a given site to migratory waterfowl can be problematic due to a number of confounding factors including the life history, habitat preferences and migratory habits of the selected waterfowl species. Additionally, regional climate conditions and anthropogenic influences can affect the timing of the annual migration and duration a species spends at a given site. The presentation will focus on study design, a review of baseline and historical data and discuss the challenges associated with the interpretation of this data in a risk-based remedial decision making framework.

WP188 Multiple Lines of Evidence to Assess Remediation and Restoration in a Mercury Contaminated River

J. Collins, B.J. Reese, AECOM; R.G. Stahl, E.I. DuPont de Nemours and Company / Corp Remediation

Historical mercury (Hg) releases occurred at a textile manufacturing facility on the South River, Virginia. These releases resulted in increased Hg concentrations in biotic and abiotic media, which have not declined over the past thirty years, as originally expected. Introduction of legacy Hg impacted soils to the South River, through bank erosion is the highest source of Hg loading to the system. Interim remedial measures targeted at reducing this source within the first few river miles are anticipated to begin in 2016. However, owing to its size, linear nature and spatial variability of the site, the remedial strategy requires that the river system be divided into manageable segments, and that remediation occurs in an upstream-to downstream fashion over a period of several years. A monitoring program comprised of multiple lines of evidence is being instituted that aims to document a reduction in Hg concentrations in a range of endpoints at differing spatial and temporal scales, in recognition of the phased remedial approach. The Short-term Monitoring (STM) program is intended to evaluate changes in Hg concentrations as well as ecological habitat quality at a specific bank segment following remediation over a relatively short time period (i.e. 2-10 years). The Long-term Monitoring (LTM) program is intended to document reductions in methylmercury exposure and improvements in habitat conditions throughout the South River and South Fork Shenandoah River at larger spatial (~160 river miles) and temporal (>10 years) scales. Monitoring data will be evaluated in an adaptive management framework that will inform remedial decision making and future monitoring efforts. This presentation will cover specific details of the monitoring plans and focus on the benefit of collecting synergistic lines of evidence that may help explain potentially confounding results in monitoring datasets.

WP189 Assessing remediation of contaminated sediments using biological endpoints: toxicity, food web tissue contamination, biotic condition and DNA damage

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The Ottawa River is a component of the Maumee, Ohio Area of Concern (AOC) as defined by Great Lakes Water Quality Agreement between the U.S. and Canada. A Great Lakes Legacy Act sediment remediation project took place in the lower 14.2 km of the river where urban and industrial activities impacted the beneficial resources of the river. Sediment was removed based on a surface weighted average concentration model where PCB and PAH levels exceeded targeted levels. Each year from 2009-2013 and again in 2015, various biological indicators were collected to assess the remedy effectiveness: fish from different trophic levels (largemouth bass, brown bullhead, white sucker, pumpkinseed, gizzard shad, bluntnose minnow, emerald shiner), macroinvertebrates (collected with multi-plate samplers) and tetragnathid spider tissue concentrations were analyzed; DNA damage was measured in brown bullhead blood; sediments were tested for toxicity using amphipod and midge 10-day survival/growth methods and macroinvertebrate biotic condition was assessed using Ohio's multimetric Lacustrine Index of Community Integrity (LICI). Sediment concentrations of PCBs immediately showed a significant decrease from pre-dredge levels. Tissue concentrations for most biological indicators increase the year following dredging and remained at pre-dredge concentrations until at least 3-years post-remediation. Gizzard shad and emerald shiner showed lower tissue concentrations 3-yrs post-dredge compared to pre-dredge across the entire project area. Five years post-dredge macroinvertebrate and spider PCB tissue concentrations were below pre-dredge levels. DNA damage in brown bullhead increased during dredging then declined in subsequent years. Sediment toxicity was reduced within 1-yr post-dredge for Hyalella azteca and 2-yrs post-dredge for Chironomus dilutus as measured by survival. No difference in the LICI was found based on samples collected before and after dredging from dredged and undredged reaches. The 5-year post-dredge fish tissue results will also be presented. The long-term tissue level targets are being met at least as quickly as the predicted 10-yr timeframe (by 2020) based on modeling performed during the project's design phase.

WP190 Estimating the Rate of Recovery in Hudson River PCB Levels: An Assessment of MNA Rates prior to the Start of Remediation

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The 2002 Record of Decision (ROD) for the Hudson River PCBs Site called for environmental dredging of approximately 2.65 million cubic yards of PCB-contaminated sediment that began in 2009. Therefore, the long remedial design (RD) period (2002-2008) provided the opportunity to collect annual data on PCB levels in water and fish for a long time interval, thus representing monitored natural attenuation prior to the start of dredging. These data provided a basis whereby the estimated rates of recovery incorporated in the ROD could be examined in light of the new data. In addition, a synoptic study of PCB levels in sediment was also conducted during the RD period. The analysis of these data provided important verification of the predicted rates of decline for PCB levels in fish and water. For the sediments, however, the new data challenged the accuracy of the original studies completed for the ROD. PCB levels at 3 different water column monitoring locations in the Upper Hudson were shown to decline at approximately 10 %/yr, consistent with what was anticipated by the ROD. For fish tissue, the data set was quite extensive, covering up to 9 species across 2 freshwater Upper Hudson and 4 tidally-influenced Lower Hudson stations. For the Upper Hudson and furthest upstream Lower Hudson stations, lipid-normalized fish tissue burdens declined at a rate of approximately 9 %/yr (8-year half-life), consistent with ROD expectations. These rates of decline were observed across species representing multiple trophic levels and environments, including young-of-the-year and adult specimens. For the 3 remaining Lower Hudson stations, rates of decline decreased with distance from the Upper Hudson, with fish body burdens at the farthest downstream Lower Hudson station exhibiting little decline with time. In a parallel manner, concentrations in the Lower Hudson fish also decline with distance downstream from the Upper Hudson. The decreasing rates of decline in the Lower Hudson suggest diminishing influence of Upper Hudson conditions on the Lower Hudson. While rates of decline for PCBs in both fish and water column data are internally consistent, the estimated rates of decline obtained by comparison of 3 synoptic sediment surveys were not. This presentation will compare the sediment, fish and water results and highlight the difficulties in using generic sediment sampling as a basis to track recovery of impacted sites through time.

WP191 Integrating Unmanned Systems into Remediation and Restoration Practices

P. Martin, NewFields

The emergence of new technology is placing powerful data acquisition tools within reach of scientists and engineers engaged in remediation and restoration activities. Three diverse case studies are presented that highlight the utility of small Unamanned Aircraft Systems (sUAS) and Unmanned Surface Vessels (USV) in rapidly and effectively measuring key parameters which may be used to evaluate project sites on a scale previously not attainable with one or two staff technicians. These examples include: (1) Recurrent aerial imagery, topography, and video to evaluate the success of mainstem and side-channel salmon habitat restoration; (2) Combined sUAS topography and USV bathymetry to evaluate the feasibility of restoration alternatives for legacy mine sites; and (3) USV cross-section elevation profiles to inform a hydraulic model of in-stream chemical biodegradation. The common thread among this diverse range of projects is that all data were collected and processed in-house by staff scientists or engineers engaged in the projects. Furthermore, if not for the reduced cost, ease of mobilization, and low response time of the unmanned systems, the datasets would not have been collected, and the sites would not have benefited from critical information. Several themes will be highlighted through discussion of the case examples: The versatility of unmanned platforms creates new options for monitoring complex sites with challenging or unsafe access. Combining traditionally separate data types through a common workflow (e.g. multi-parameter aerial and aquatic surveys) leads to a synoptic understanding of processes occurring at a site by introducing multiple lines of evidence to support observations. Commercially available turn-key systems help to simplify mission planning and data acquisition, allowing researchers to focus on the problem rather than the details of operating the equipment. Unmanned tools are easily integrated into existing data-management systems by utilizing standard software to process and manipulate sensor-specific or geospatial data.

WP192 Engineered plant for phytoremediation of environmental contaminant-the current state of affairs

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Phytoremediation has been described as an efficient medium through which chemical hazards that can be identified by various classes of pollution could be removed from the soil; hence it suffers various limitations that has prevented the field application of the technique. Plants lack the metabolic enzymes required for full pollutant remediation and this often results in slowing the pace at which phytoremediation activity occur. Such inherent limitation of plants for complete remediation of xenobiotic compounds calls for the idea to genetically modify plants using bacteria or mammalian genes that take part in the degradation of chemical compounds that are found to be toxic. Various plants have been implicated in this new line of biotechnology. Whilst most of them defile the inherent limitations, others are affected by the challenges and therefore are unable to achieve the primary goal which is environmental pollution remediation. This study provides an in-depth analysis of various endophytic-transgenic phytoremediation studies on organic contaminated environment. It also highlighted the kind of plants employed in such application, the strength and weakneses of the plants as regards the contaminant in question as well as the measure of remediation that were used. The study proffers better alternative plants for phytoremediation of organic chemical contaminants based on the type of contaminant and the intending remediation protocol to be followed.

WP193 A Comparison of Passive Sampling and Bioaccumulation Measurements in the Evaluation of Bioavailable Concentrations of Pesticides Impacted Sediments

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Bioavailability of contaminants in sediment is an important consideration in the determination of potential risk of adverse effects from exposure to benthic invertebrate. Numerous tools are used to assess bioavailability, including measurement of contaminants in tissue via bioaccumulation testing and sediment porewater with passive sampling. These tools and different methods of application vary in cost effectiveness, efficiency, and ability to incorporate field conditions. In this presentation, the performance a thin-layer habitat enhancement sand cap (target depth 6 inches) to reduce bioavailable concentrations of organochlorine pesticides (e.g., 4,4'-dichlorodiphenyldichloroethane [4,4'-DDD] and 4,4'-dichlorodiphenyldichloroethylene [4,4'-DDE]) in sediment was evaluated at Site 99 Quantico Embayment in the Quantico Marine Corps Base, Quantico, Virginia, USA. The thin-layer cap placement is expected to reduce concentrations of total DDX in sediment more rapidly than natural recovery processes alone. Prior to and following installation of the cap, monitoring at the site included three tools to assess bioavailability. Concentrations of pesticides in tissue were obtained via two-week deployment of in situ bioaccumulation testing with Sediment Ecotoxicity Assessment (SEA) Rings using two species, Lumbriculus variegatus (oligocheaete worm) and Corbicula fluminea (Asian clam). Secondly, a two-week deployment of in situ solid phase microextraction (SPME) passive sampling was used to measure porewater from 0 to 24 inches below the sediment-water interface. Lastly, ex situ SPME was also used to measure porewater at multiple depth intervals within and below the cap. Strong correlation was also observed between the in situ and ex situ passive sampling in concentrations of total DDX in surface sediment porewater ($R^2 = 0.88$). Also, strong correlation between concentrations of total DDX in wormtissueandsurface sediment porewater as measured byboth in situ and ex situ passive sampling approaches was observed ($R^2 = 0.82$ and 0.95, respectively). However, concentrations of total DDX in clamtissueandsurface sediment porewater as measured byboth in situ and ex situ passive sampling approaches was found to be poor ($R^2 = 0.28$ and 0.16, respectively), likely due to the filter feeding clam being exposed to overlying water rather than sediment porewater. Further evaluation between these bioavailability assessment tools as well as advantages and disadvantages will be presented.

WP194 Assessing Sediment Remedial Status in Three Lake Ontario Areas of Concern

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Contaminated sediment has been identified as one of the major impediments to the remediation of many Areas of Concern (AOCs) in the Great Lakes. Three such AOCs within the Lake Ontario watershed were investigated in this project: Metro Toronto Harbour region, Hamilton Harbour, and Lyons Creek (Niagara River). Each AOC presents a unique set of beneficial use impairments in relation to sources and type of sediment contamination, and each has a unique Remedial Action Plan geared toward beneficial use improvement and eventual delisting. In this study, a sediment quality tetrad (SQTet) was applied in a weight-of-evidence assessment to evaluate current sediment contamination for these AOCs and to compare the current state to historical contamination levels and impacts to determine if improvements have occurred. The SQTet included assessment of bulk sediment chemistry, toxicity testing, benthic community analysis, and bioaccumulation. Results to date based on chemistry and toxicity show continued concern surrounding PAHs in Toronto and Hamilton Harbour sediments, with at least one site elevated above the severe effect level for Provincial Sediment Quality Guidelines in Toronto, and all three exceeding in Hamilton. Hamilton Harbour sediments resulted in growth and reproductive impairments in chronic laboratory exposures, as well as elevated PCB tissue concentrations. Sediments from Humber Bay in the Toronto AOC resulted in growth and reproductive impairments, as well as high tissue levels of PFCs in laboratory bioaccumulation exposures. Lyons Creek sediments resulted in acute toxicity and elevated tissue concentrations of PCBs and metals. These data indicate that there has been limited improvement for the sites investigated, and that present delisting targets may need to be revisited.

WP195 Using tree swallows to assess remedy effectiveness at Great Lakes Areas of Concern (AOCs), 2010-2015

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Tree swallows (Tachycineta bicolor) are being sampled across the Great Lakes basin as part of the Great Lakes Restoration Initiative (GLRI) to provide a system-wide assessment of current exposure to organic contaminants. At selected sites dredging has been done, is ongoing, or is planned. Tree swallows, because their diet is mainly the aerial stage of benthic aquatic insects, offer a unique opportunity to assess the effectiveness of sediment removal on contaminant uptake in biota. Remedy effectiveness is monitored before and after sediment removal. When swallows migrate away from nesting areas, they depurate accumulated contaminants. Thus, when they return in the Spring, they start with virtually a 'clean slate,' and changes in bioavailability of contaminants at the Area of Concern (AOC) can then be assessed by analysis of eggs and nestlings. Three metrics, contaminant concentrations in eggs, contaminant concentrations in nestling carcasses, and the rate of accumulation in nestling carcasses can be measured. At Lincoln Park, Milwaukee Estuary AOC, tree swallows were monitored for 3 years pre-dredge and for 2 years post-dredge. Approximately 119,000 cubic yards of sediment were removed during Phase 1 of the cleanup effort. For PCB egg concentrations, while there was not a significant change in exposure pre- (1.53 mg/g wet wt.) vs. post-dredging (1.02 mg/g wet wt.) there was a declining trend. PCBs in nestlings (1.33 mg/g wet wt. pre-dredging vs. 0.74 mg/g wet wt. post-dredging) and accumulation rate (2.30 mg/day pre-dredging vs. 1.12 mg/day post-dredging), however, were significantly lower after dredging. Trends in egg exposure were more variable than nestling exposure, which may have contributed to the non-significant response observed in eggs. Data from other sites will be presented with different dredging scenarios.

WP196 Long-Term Monitoring of Remedy Effectiveness for Study Area 7 in Upper Newark Bay

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This presentation discusses the long-term monitoring program of remedy effectiveness for approximately 37 acres of capped sediment located on the eastern shore of the Hackensack River in Upper Newark Bay adjacent to a site known as Study Area 7 (SA-7). Chromium ore processing residue (COPR) was placed as fill material to create what is now SA-7. Following several phases of investigation, the U.S. District Court approved a Consent Order requiring the remediation of sediments within the Hackensack River. The specified sediment remedy included dredging a 0.5-acre area adjacent to the SA-7 bulkhead, capping of approximately 37 acres (divided into 30 cap areas), and monitored natural recovery of approximately 33 acres, as well as requirements for a long-term monitoring program (LTMP). The dredging and capping of sediments for all but three of the cap areas were completed between July 2012 and October 2013. The long-term monitoring program was initiated immediately following remedy implementation, and is now in its third year. The LTMP will span 15 to 25 years, with monitoring activities taking place in Years 1, 2, and 5 and in 5-year intervals after Year 5. The monitoring activities of the LTMP are based on the following objectives defined in a Consent Order: ensuring that the integrity of the caps is maintained; confirming that areas of MNR are not scoured out; and collecting data regarding the nature of the benthic community in remediated sediments. The monitoring completed in Years 1 and 2 of the LTMP included hydraulic and hydrodynamic evaluations, bathymetric surveys, cap integrity monitoring, pore water sampling, sediment profile imaging (SPI), and biological sampling. To date, the monitoring program has demonstrated that the remedy remains stable and shows an improvement in the benthic community relative to as-built conditions. Bathymetric survey results generally show a net deposition of sediment across both the sediment cap areas and the MNR areas. Cap inspections confirm that the armoring of the caps remains in-place with no evidence of erosion of cap materials. SPI survey and biological sampling results indicate development of an established and diverse benthic community in both the cap areas and MNR areas. In addition, the planned methods of verification have been successfully implemented such that no changes in procedures are necessary.

WP197 Recommendations for the Use and Development of Biota-Sediment Bioaccumulation Models for cPAHs

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Paired sediment and tissue data are used at contaminated sediment sites to develop biota-sediment accumulation factors (BSAFs) and biota-sediment accumulation regressions (BSARs), which are models of statistical relationships between concentrations in sediment and tissue. These models can be a key factor influencing sediment remedial decisions at sites where sediment remediation goals back-calculated from tissue concentrations drive unacceptable risks to humans and the environment. This paper evaluates the current practice of developing BSARs and BSAFs (BSAR/Fs) for carcinogenic polyaromatic hydrocarbons (cPAHs) and, based on an independent evaluation of co-located data, makes recommendations for developing cPAH BSAR/Fs in the future. The development of BSAR/Fs for cPAHs is more challenging than for other organic chemicals because PAH metabolism and site-specific factors affect the bioavailability and bioaccumulation of PAHs. Available cPAH BSAR/Fs found in the literature and at sites nationwide are highly variable; the range in values indicates that sites are potentially using inconsistent methods and assumptions to develop a sediment-tissue relationship, and illustrates the need for a consistent, defensible methodology. Uncertainties associated with BSAR/Fs have major remediation cost implications. Because of this, pragmatism is needed when evaluating, and if necessary, mitigating cPAH risks to people who eat fish and shellfish. First and foremost, if a suitable relationship cannot be developed using site-specific data, BSAR/Fs should not be used. If a site-specific BSAR/Fs can be developed, then the uncertainty associated with the BSAR/Fs and the reliability of risk estimates or remedial action levels (RALs) calculated using the BSAR/Fs must be seriously evaluated to understand the level of certainty that is inherent in this analysis. As the level of uncertainty increases and remediation costs escalate, alternative methods discussed in this report should be used to set risk management objectives and implement risk management actions.

WP198 Risk Assessment and its Role in the Remedial Design Process

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Risk assessment is a standard tool employed during the remedial investigation (RI) phase for a contaminated site. Conservative and generic assumptions are often used in risk assessments, and this is done to insure that risks are not underestimated and health protectiveness is achieved at a site. However, such assumptions may not be wholly appropriate for a given site, or for separate portions of a site. This is especially true in the case of "screening-level" risk assessments. Risk results are then carried forward into the feasibility study (FS) and in most cases never refined or reviewed for their applicability, especially as the site is "carved out" into remedial decision units. Remedial goals, remedial action levels, and remedial footprints are then estimated using these initial risk assessment assumptions. Furthermore, the RI/FS process is often a prolonged process requiring multiple years to complete. If site conditions change (e.g., an interim measure is implemented involving removal of contaminated source material), the original risk assessment assumptions may no longer be valid, meaning that less remediation might presently be required. For large and complicated contaminated sediment sites such as ports and large rivers, the RI/FS phase is likely to take up to a decade or more, so changes in site conditions are common and can be significant in terms of their impact on the final design of the remedy. Changes may occur in sediment and biota concentrations; land use, the numbers and types of human and ecological receptors present; and even in what is known about the toxic effects of certain chemicals. To manage the potential for change, flexibility should be written into the FS, proposed plan, and record-of-decision to allow for necessary updates in the risk assessment which may cause the need for changes in the pre-design and design phases. Salient examples from risk assessment case studies describing instances will be presented where such flexibility was necessary given greater site-specificity or changes in site conditions related to chemicals of concern; exposure conditions; site-specific and applicable receptors; bioaccumulation and new information on chemical toxicity.

WP199 Use of multivariate analyses of macroinvertebrate community data to inform monitoring and remediation efforts: Niagara River tributaries case study

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Multivariate analyses specifically designed for ecological community data, such as nonmetric multidimensional scaling (NMDS), are useful to show the degree of similarity between communities and to determine if abiotic factors correlate with differences in community structure. A multi-agency (USEPA, NOAA, NY State DEC, USGS, USACE) study of 7 tributaries to the Niagara River Area of Concern (AOC) is measuring levels of polychlorinated biphenyls (PCBs) and polyaromatic hydrocarbons (PAHs) in the sediments and macroinvertebrates while collecting macroinvertebrates for community analyses. Also, the study is collecting PCB and PAH concentrations from mussels and passive samplers as part of a weight of evidence approach. To inform remediation efforts, ranking sites in terms of community metrics (e.g., HBI, taxa richness, tolerance values) and levels of contaminants of concern has provided useful information. We use NMDS and multi-response permutation procedures (MRPP) as additional tools to explore the complex community data. For example, part of this study compared two different Hester-Dendy (HD) sampler designs. An NMDS analysis was performed on the species abundance data for two HD sampler designs, which were co-located (attached to the same mooring), except that the Design 1 HDs were attached near the sediment, and Design 2 HDs were suspended in the water column. NMDS graphs showed that the communities retrieved by the 2 designs were similar in many cases (grouped together in 2D and 3D space), but for a few sites they were far apart. When the data for the co-located pairs of samplers that did not group together on the NMDS graphs was examined in more detail, it was found that for these few sites Design 2 HDs had greatly reduced taxa richness. Also, chironomids, which are fairly ubiquitous, were not retrieved at all. The coefficient of variation for taxa richness for Design 1 was lower (22%) than Design 2 (34%), likely due to these few site samplings. As there was no pattern with regard to variables like site location or water depth, the cause is likely some type of more random disturbance related to Design 2 HDs' position in the water column. We also use multivariate community analyses to enhance the process of ranking multiple tributary sites in terms of their disturbance, and to investigate correlations between PAH & PCB contamination and macroinvertebrate community assemblages.

WP200 Field sampling and laboratory evaluation of reactive capping/in situ treatment of mercury contaminated solids using DGT devices

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A major challenge in remediating contaminated mercury sites is the fact that the inventory of bulk mercury in sediments often does not relate directly to exposure and risk in organisms of concern. Much of that mercury may be largely immobile and unavailable for methylation. An alternative to traditional bulk mercury measurements is the use of Diffusive Gradient in Thin-film (DGT) profilers that measure the mobile and most available forms of mercury in porewater. Measurements are based on the rate of diffusion of mercury species through a diffusive layer of known thickness onto a resin that acts as an effectively infinite sink. DGT profilers are able to provide depth specific measurements of mobile and potentially available mercury and can be used to assess methylation potential through simultaneous measurement of reducing conditions via voltammetry. In this paper, DGTs are used to assess exposure and transport mechanisms as well as the effectiveness of potential remedial options for mobile and available mercury at a contaminated sediment site in South River, VA. DGT samplers were deployed in river bed and bank sediments for field assessment and in laboratory mesocosms to evaluate potential remedial approaches. The field sampling efforts suggested that leaching of non-particle associated mercury from bank sediments during bank drainage after flooding events was a potentially important mercury source. Simultaneous measurements of methyl mercury and reducing conditions suggested that the mercury was primarily inorganic mercury. Under normal (i.e. non-draining) conditions, there was greater evidence of reduction and methylation but less direct exchange between bank waters and the river. The mesocosm efforts were designed to evaluate potential remedial approaches that would address this mercury release mechanism including the addition of layers of sorbents to more strongly retain the mercury. Biochar, activated carbon, and layers of natural sediment were all considered for these sorbing layers. A granular activated carbon layer was shown to be the most effective in the removal of total mercury and methylmercury from the sediment under the reactive cap.

WP201 Evaluating the toxicity of Pine River Sediments Downstream from the Velsicol Superfund Megasite, St. Louis, MI

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Downstream of the Velsicol Superfund site has had historic contamination of numerous chemicals including DDT, PBB, and HBB among others. Remediation and sediment dredging near the site began in spring of 1999, and was completed in 2006. In 2011, the Michigan Department of Environmental Quality completed a Baseline Assessment Report Long Term Monitoring Plan for the Pine River. This report revealed that downstream from the Velsicol Superfund site, there were several locations with levels of DDT that were still greater than 50 parts per million. However, there has been limited assessment of the benthic community since this evaluation. The objective of this research was to determine if Pine River sediment downstream from the superfund site was still toxic to aquatic macroinvertebrates after decades of degradation and removal of the source via dredging. Three sites were selected downstream and an upstream reference site was used. At each site, macroinvertebrates surveys were conducted and sediments were collected for laboratory toxicity tests for mortality and sublethal effects using Hyalella azteca, Chironomus dilutus, and Hexagenia spp. This study indicated that the most severe impacts may be limited to areas within a few miles downstream. However, more research is needed to evaluate potential effects on ecosystem function and other riparian species.

Fate and Effects of Chemicals from Diffuse Sources and Stormwater – Poster Only

WP202 Developing Low-Cost Stormwater Sampling Equipment Using Programmable Microcontrollers

R.E. Casey, Towson Univ / Chemistry

Stormwater issues are gaining regional, national and international prominence as regulatory agencies, water utility practitioners and academic researchers grapple with the quantity and quality of stormwater in developed landscapes. There is a need for better understanding of stormwater chemistry and the contributions of these dynamic events to pollutant transport, impacts on biota and regulatory compliance. At present, efforts to gather stormwater data involve a compromise between personnel costs, equipment costs and sample analysis costs. Typically, this compromise leans heavily toward personnel and equipment costs, limiting the amount of actual data collected. This project seeks to shift the balance toward data acquisition, enabling more comprehensive stormwater sampling campaigns that can address the chronic shortage of reliable stormwater data and promote a more data-rich context for stormwater decision-making. Low-cost programmable microcontrollers can be utilized to create stormwater sampling equipment that can automate sampling campaigns at a fraction of the cost of commercial equipment. These platforms can also allow for project-specific customization of sampling functions that is not possible with commercial equipment. This effort utilized the open-source Arduino programmable microcontroller to generate a composite stormwater sampler capable of flow-weighted composite sampling when coupled with an impeller-based flow gauge or with a tipping bucket flow gauge. The system incorporates a data logger to record flow and sampling events at customizable intervals for each data type. Comparable commercial equipment can cost US$900 – US$2000 per sampling location but this system was built for US$115 and could be reproduced for US$70 per sampling location, while meeting or exceeding the performance requirements for use in research. Low-cost automated stormwater sampling can decrease personnel costs, decrease risks to personnel during storm conditions (lightning, high water conditions) and increase sampling frequency and spatial resolution. Low-cost equipment can also open stormwater sampling to citizen-science groups including watershed organizations and schools. The utilization of open-source components and the ability to easily disseminate and edit the code mean that this prototype can be readily reproduced, optimized and adapted to the needs of specific sampling projects.

WP203 Combined effects of water hardness and stormwater contaminant toxicity to Daphnia magna

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Regulatory concerns have been expressed regarding diffuse and non-point sources of copper (Cu) released by Cu roofs. Copper roofing materials can contribute much higher mass loads of dissolved copper per unit area than other surfaces such as parking lots and roadways. Bioretention structures such as planter boxes, swales and rain gardens are being increasingly utilized in built landscapes as a strategy to attenuate both storm water flows and contaminant loads. In previous studies, storm samples were collected before and after treatment through bioretention structures and tested for toxicity with Daphnia magna. Daphnia magna live naturally in hard and moderately hard waters. Storm samples are typically of very low water hardness, so in order to remove the low ion stress during testing, Daphnia were acclimated in intermediate hardness water (62 mg/L $CaCO_3$). Acclimated Daphnia survived 48 h acute testing, whereas Daphnia raised in moderately hard water (83 mg/L $CaCO_3$) died when exposed to control stormwater. We have conducted further studies of intermediate water acclimated Daphnia related to stormwater testing conditions. Intermediate water cultured Daphnia as well as hard (165 mg/L $CaCO_3$) water cultured Daphnia were tested for reproductive success in a chronic 21-day experiment. Daphnia were exposed in a 2x2 design where < 48 h old F1 generation neonates (n=10) from parents raised in intermediate water were placed individually in 40 mL of intermediate or hard water, and < 48 h old F1 generation neonates from parents (P1 generation) raised in hard water were also evaluated for reproductive success. Feeding and water renewal occurred 3 times weekly, and reproduction data was collected daily for 21 days. Results showed the highest offspring production for the P1 Hard x F1 Hard combination, followed by P1 Intermediate x F1 hard, then P1 Hard x F1 Intermediate, and lastly P1 Intermediate x F1 intermediate combination. In addition to lower offspring production, Daphnia tested in intermediate water showed longer time to first brood. With this information, further testing will be conducted to evaluate the stress of low hardness waters on Daphnia reproduction and response to chemical stressors. Additional chemical stressors will be evaluated in acute and chronic tests to further understand the relative sensitivity of Daphnia raised in lower ionic conditions.

WP204 Sodium, Calcium and Chloride Removal Efficiencies of Self-Converted Dry Detention Ponds in Baltimore County, MD

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Road de-icing materials, such as road salt (NaCl and $CaCl_2$) and brine, have become common practices for pre and post winter storm application in Maryland. High salinity runoff goes either directly into streams or into stormwater ponds. Stormwater ponds are a common best management practice (BMP) for the management of runoff from impervious surfaces in suburban and urban landscapes. These ponds range from constructed depressions designed to detain and slow stormwater runoff to allow some sedimentation to occur or can be deeper more engineered structures that hold water year round. Stormwater BMPs have shown to be beneficial for nutrient removal, but what about for de-icing materials in the winter. In this study, ponds designed to have short hydro-periods, and therefore be dry most of the time, were evaluated for their Na, Ca and Cl removal efficiencies. Over time, these ponds can convert from “dry” ponds to ponds that have wetland characteristics (soils, plants) and may also hold water for longer periods of time. This study collected data from six ponds (three control and three self-converted), between August 2014 and October 2015. Continuous water level sensors were installed for continuous flow records at each inlet and outlet and during selected rainfall events, grab samples were collected at inlet(s) and outfalls during the rise, at the peak, and during the falling limb of the hydrograph. Grab samples were analyzed for Na, Ca and Cl. Ion loading and removal efficiencies are based on flow-weighted event mean concentrations. With an increase in impervious surfaces in Maryland and the need to use more de-icing material during winter storms, freshwater streams and groundwater are increasing in salinity causing stress to organisms and everlasting effects on the ecosystem. This study showed that in all six ponds, there was an increase in loadings and concentrations leaving the pond. The analysis suggests that stormwater BMPs may be concentrating Na, Ca and Cl inside the ponds and releasing high concentrated water in pulses. Changes will need to be made with how urban and suburban stormwater is treated if we want to keep our freshwater systems fresh.

WP205 Preliminary Evaluation of Alternative Toxicity Testing Methods for Episodic Discharges Including Stormwater

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Whole Effluent Toxicity (WET) tests for the monitoring of continuous point source discharges are highly standardized and typically include static exposure of an undiluted end-of-pipe sample over a period 4-7 days, depending on the toxicity endpoint. These standard WET test protocols overestimate the exposure conditions of non-continuous discharges such as episodic storm water runoff events, periodic short-term applications of pesticides, short term spills, or other ephemeral or time-varying pulses of contaminants. This suggests that these tests potentially overestimate the toxic effect of these types of discharges. We investigated an approach to modifying WET testing procedures to account for the time-varying aspect of the exposure condition. We believe that these procedures have the potential to improve the ecological relevance of the testing outcomes, particularly as they are applied to compliance monitoring. We conducted side-by side acute and chronic toxicity tests using both a standard 96-hr static test exposure protocol and a modified pulsed exposure condition. Chronic toxicity tests were conducted by exposing purple sea urchin (Strongylocentrotus purpuratus) embryos and mysid shrimp (Americamysis bahia) to laboratory seawater spiked with copper and/or zinc at varying concentrations. The pulsed tests used exposures of 3, 6, or 12-hrs followed by transfer to clean seawater for the remainder of the 96-hr test. The laboratory testing procedures were designed to simulate common contaminants and discharge durations found in San Diego storm water runoff. Additionally, these testing protocols were evaluated on multiple samples of storm water runoff collected during a single rain event in San Diego. Initial results indicate that pulsed exposures resulted in EC50 or LC50 values up to two orders of magnitude greater (i.e. less sensitive) than the standard 96-hr exposure conditions in both laboratory spiked samples and storm water runoff samples. The results suggest that short term pulses of copper and zinc have a lower toxic effect than is represented by standard continuous exposure testing. The presentation will summarize results to date, future research plans, and implications to compliance monitoring under National Pollution Discharge Elimination System (NPDES) permits.

The Other Oil Spills

WP206 Extreme temperature and oil contamination shape the relative abundance of copepod species in the Arctic

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The retreat of sea ice in the Arctic under global warming is predicted to intensify oil exploitation and shipping activities in this region, posing the risk of oil contamination. Knowledge on how Arctic secondary producers deal with the combined effects of global warming, particularly the extreme temperature and oil exposure is limited. To address this, we exposed females of two copepods species Calanus glacialis and C. finmarchicus to pyrene at three temperatures: 2, 6 and 10°C. Both species co-exist in the Disko Bay, Greenland, but only C. glacialis is a true Arctic species while C. finmarchicus is of north Atlantic origin. Pyrene is one of the most toxic components of crude oil to marine copepods. The temperatures of 2, 6 and 10°C represent the mean sea water temperature, the 4°C increase in mean temperature by 2100 as predicted by IPCC scenario RCP8.5 (2013) and the extreme sea water temperature, respectively, in Disko Bay. Four-degree increase in mean temperature did not have an effect on grazing rate and survival of both species. However, the extreme temperature (10°C) increased the grazing rate and mortality of C. glacialis, but not in C. finmarchicus. Exposure to high pyrene (250 nM) strongly reduced survival and grazing rate in both species and this pattern was independent of temperatures. Notably, exposure to high pyrene (250 nM) resulted in 72% of mortality in C. finmarchicus that was two times higher than the mortality observed for C. glacialis. These results suggest that extreme temperature under global warming and oil contamination may drastically change the relative abundance of pelagic copepod community by changing the species-specific vulnerability to the extreme temperature and oil exposure.

WP207 Evaluation of sublethal effects of the soluble fraction of Mexican light oil and gasoline on the oyster Crassostrea virginica

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Due to the energy reform, Mexico is increasing its efforts to obtain oil from deep water deposits, this increases the probability of having accidental oil and other fuels spills in the Gulf of Mexico, which represents a risk to important fisheries resources like oysters. The response of these organisms to oil and other fuels has not been studied in local populations of Crassostrea virginica, so the aim of this study was to investigate the effects of the soluble hydrocarbon fraction in oysters extracted in local lagoons, using biomarkers at different levels of organization. C. virginica has been used as a bioindicator organism in several studies due its ability to bioaccumulate contaminants and reflect their effects. The water-soluble fraction of Mexican gasoline Magna and light Mexican oil were obtained after shaking a 4:1 solution of fuel:water for 24 h. The bioassay included a seven days exposure period and a seven days recuperation period to assess toxicity and recuperation. A group of biomarkers were evaluated during the exposure and recovery phases: acetylcholinesterase (AChE) activity was evaluated using Ellman´s colorimetric method, while lysosomal membrane stability (LMS) was evaluated by the retention time of neutral red. In the exposure phase, significant differences were observed in LMS and AChE, associated to concentration and time. The exposure to gasoline indicated a partial recovery, however exposure to light oil showed damage without recovery. C. virginica is recommended for the monitoring of pollutants in the coast of the Gulf of Mexico due its ability to reflect the sublethal effects of these pollutants. AChE and LMS can be used as biomarkers of response in aquatic organisms in short-term exposures to hydrocarbons.

WP208 Identifying toxic components in Iranian Heavy crude oil on zebrafish (Danio rerio) embryos by sequential fractionation

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Crude oil is a complex mixture composed mainly of aromatic and alkylated hydrocarbons. Identification of key components that exert biological effects in crude oil is important. We employed effect-driven analyses to identify the key components in crude oil that exert adverse effects such as malformation of fish. A series of fractionation techniques were used to separate Iranian Heavy crude oil; 33 fractions by true boiling point, further 3 subfractions by silica gel column, and (into further 3 subfractions), and even further 10 secondary subfractions by HPLC. Developmental outcomes (mortality, hatchability, and time to hatch) and malformation (yolk sac and heart edema) were observed in zebrafish (Danio rerio) embryos following 120 h exposure to each distilled fraction. Only four fractions, containing mostly high molecular weight compounds, showed toxicological effects. Following further fractionation by polarity using silica gel column, aromatic and polar/resin compounds, that include mostly alkylated dibenzothiophenes and phenanthrenes, induced significant malformation (heart edema). When these subfractions were further separated by HPLC according to their logKow values, malformation incidences generally decreased. This observation indicates that malformation in fish was caused not by small group of chemicals but by interactions of multiple constituents of oil. The subfractions that showed increased malformation in fish, as well as their secondary subfractions showed higher arylhydrocarbon receptor (AhR)-mediated potencies in H4IIE-luc AhR binding affinity assay. Overall, our results indicate that components of crude oil that include alkylated dibenzothiophenes and phenanthrenes may interact with one another to exhibit toxicity on teleost development, perhaps via AhR-independent pathway. This work was supported by Korean Ministry of Oceans and Fisheries Project PM56951.

WP209 Air Exposures Following Oil Spills – An Overview

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Oil, when spilled in the oceans, emits volatile and semi-volatile compounds at the ocean surface which dissipate quickly. Organic aerosols are also emitted, and can transform into secondary organic aerosols in the air. These particles have the potential to be transported over longer distances. Air exposures for workers, for shoreline communities, and for wildlife may occur, depending on the size of the spill, the locations of off-shore workers at the time of the spill, the distance of the spill from shore, meteorological conditions, and oil type. Air concentration data were collected by a variety of groups following the Deepwater Horizon oil spill, but were neither easily accessible nor were collated in one database. In addition, the various groups collected air data using different sample collection methods, and different laboratories and analytical methods. Importantly, air concentrations at heights above the water and locations that would be relevant for health risk assessment, for example, for marine mammals and for workers, were not consistently measured. Clearly, the air concentrations to which humans and wildlife may be exposed following both larger and smaller oil spills is not well-quantified. We outline the modeling processes that would be necessary to accurately estimate air exposures for risk assessment. As examples, we present air fluxes from small, medium, and large oil spills, and discuss how these fluxes can be used to estimate exposures for workers, both off-shore and on-shore, and for coastal communities.

Experimental and Modeling Approaches to Account for Real-World Complexity in Environmental Toxicology

WP210 A Commercial Agricultural Product, Borregro HA-1, Decreases Bioavailable Copper to Daphnia ambigua and Ceriodaphnia dubia

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In an aquatic system, Dissolved Organic Matter (DOM) provides macromolecules which strongly attract cations such as copper, lead, zinc, and mercury. It is possible, therefore, to take advantage of DOM for natural remediation of metals pollution, as the DOM will bind with metal cations and reduce their bioavailability for uptake by aquatic organisms. Here, we describe the effectiveness of a commercially-available agricultural humate product, Borregro HA-1, for the reduction of copper toxicity in a simulated point-source discharge. These experiments were conducted to support the operation of an engineered delivery system that uses real-time flow rate and pH data to deliver the Borregro HA-1 directly into the wastestream. The system's control equation is based on the Biotic Ligand Model (BLM) for copper. Effectiveness of the Borregro HA-1 was tested through a series of acute and chronic bioassays using the indicator organisms Daphnia ambigua and Ceriodaphnia dubia. Borregro HA-1 was added to simulated outfall water with very low hardness (< 15 mg/L as $CaCO_3$) and a pH of 7.05. Borregro HA-1 was added to water the to achieve a final dissolved carbon concentration of 2.62 mg C/L, the in-stream carbon concentration calculated using engineered system's control equation when run at this pH. The 48-hour LC_{50} values for the copper plus Borregro HA-1 tests were 35.6 and 22.5 µg Cu/L for C. dubia and D. ambigua, respectively. In the tests with copper only (no Borregro HA-1 addition), the LC_{50} values were lower than the lowest test concentration (3.12 µg Cu/L) and could not be calculated. Acute results, therefore, indicated that the addition of the product to the simulated wastewater effectively diminished the toxicity of copper. Chronic toxicity tests included concentrations that would reflect the Final Acute Value (FAV; 82 µg Cu/L), the Chronic Maximum Concentration (CMC; 41 µg Cu/L), and the Criterion Continuous Concentration (CCC; 25 µg Cu/L) as determined by the BLM. Chronic toxicity test results indicated that, at a minimum, the simulated outfall water with Borregro HA-1 responds as expected to added toxicants such as copper. This could not be said for the simulated outfall water without the Borregro HA-1, where copper is much more toxic without the additional DOC in the test medium.

WP211 A Risk Assessment Strategy for a Former Shooting Range in a Dynamic Estuary

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At former shooting range sites, spent lead shot can occur as either intact or weathered particles, or as ionized salts. The challenge at these sites involves integrating ecological exposures to different forms of lead (shot and non-shot), particularly to birds relying on grit to aid in digestion. Intentional ingestion of lead shot as grit by birds can be a critical ecological exposure pathway at these sites. Incidental ingestion of lead by birds and wildlife via diet and direct contact of invertebrates to soil and sediment lead may also pose a potential risk of adverse effects. An ecological risk assessment will be performed to evaluate the characteristic ecological exposure pathways involving lead (shot and non-shot) at a former shooting range site in a dynamic coastal estuary. In order to mitigate waterfowl exposures to lead shot, the remedial action for the site involved removal of lead shot from the intertidal and subtidal area sediment through physical processing of sediments. Subsequent erosional impacts to the sediment and the winnowing effects due to differential densities of lead shot and sediment substrate have resulted in limited sporadic patches of concentrated lead shot in these remediated areas. Currently, hazing and small scale removal of lead shot are in place to limit the waterfowl exposure to lead shot in these patches. A Living Shoreline pilot study at the site has shown that installation of artificial reef balls not only reduces ongoing erosion, but also promotes sediment accretion, effectively mitigating the waterfowl exposures to lead shot. The ecological risk assessment provides a basis for monitoring the effectiveness of the Living Shoreline to reduce ecological exposures to lead at the site. The approach and findings of the ecological risk assessment, in the context of supporting the on-going and future remedial and/or risk decisions at the site will be presented.

WP212 An individual based model of larval yellow perch to link behavioral effects of methylmercury to adverse population outcomes

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Historically, yellow perch were highly abundant throughout the shallow areas of Lake Michigan; however their populations have experienced a decline since the early 1990's. The poor recruitment has been associated with changes in trophic structure and adverse environmental conditions; however the possible effects of contaminants have not been explored fully. Methylmercury (MeHg), a persistent contaminant found in the Great Lakes, has been shown to alter foraging and predator avoidance behaviors of larval yellow perch and these behaviors could affect overall growth and survival. We adapted an individual based model (IBM) to incorporate laboratory derived MeHg effects on a larval yellow perch cohort. The model was calibrated to simulate real-life situations in Lake Michigan and nearby Crystal Lake (Benzie County, MI), and varied community structures to accommodate multiple stressor scenarios. Our model simulations suggest that exposure to neurotoxic contaminants such as MeHg, when coupled with low food availability and suboptimal water temperatures, could significantly reduce cohort survival. These findings suggest that contaminants could be another factor impacting recruitment of larval yellow perch in Lake Michigan, and that this deserves further exploration.

WP213 Application of a Novel Groundwater-to-Surface Water Model to Support Ecological Risk Assessment at a Former Oil Refinery

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Long-term water levels and free extractable petroleum hydrocarbon (EPH) recovery volumes, along with dissolved concentrations of EPH's in upland groundwater and sediment porewater, have indicated possible exposure of aquatic biota to upland contaminant sources at a former oil refinery in Western Canada. A novel mechanistic groundwater-to-surface water contaminant fate and transport model (GSM) was developed to provide one line of evaluation in a multiple lines of evidence approach adopted for risk assessment at the site. The GSM represented dynamic linkages between the surface water and groundwater, taking into account variable density flow, and tidal exchange and dynamics. Two-dimensional cross-sectional models were developed for three representative cross-sections that intersected potential areas of concern identified from site investigations, which included the collection of high-frequency temperature data through a distributed temperature sensor (DTS) network to identify potential seepage zones, and collection of porewater chemistry data from prospective seepage areas in the inter-tidal zones to identify priority contaminants. The GSM was calibrated to site-specific hydrogeological data and was able to reproduce seasonal and tidal patterns in historical groundwater level measurements in the different hydrogeological units. The GSM was applied to evaluate contaminant migration over the long term to assess attenuation within the surficial sediments due to tidal exchange and degradation processes. Contaminants of concern simulated differed considerably in mobility and degradation potential. The GSM provided a means for estimating dissolved contaminant concentrations across different environmental compartments including groundwater, sediment porewater, and surface water. Model simulations indicated that biodegradation could be an important factor in reducing contaminant levels in the transition zone for certain contaminants. Moreover, the contaminant migration paths identified in the GSM were consistent with porewater contaminant data and GSM-predicted seepage patterns were consistent with those identified from the DTS study, thereby providing additional lines of evidence for refining the conceptual site model. In conjunction with the other site investigations, the mechanistic modeling results were used to identify contaminants that are likely to persist in the sediments and integrated into the ecological risk assessment for the site.

WP214 Assessing effects of harbor contaminants on snails in situ

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Many contaminants are being released continuously into water bodies. Thus it is important to assess the long term, sublethal effects of contaminants on biota, as they can be early warning signals for effects at population level. Field experiments are particularly valuable, as they account for natural variability and environmentally relevant concentrations of pollutants. In this study we chose leisure boat harbors as typical sites of contaminant accumulation (e.g. metals from antifouling paints), due to their enclosed design and poor water mixing. The test organism was the nerite snail Theodoxus fluviatilis, a highly abundant and representative species of the area studied. The aim was to investigate both mortality and histopathological effects of harbor contaminants on snails, as well as the importance of contaminant pre-exposure on the sensitivity of snails. We exposed two different populations of snails (i.e. 'clean' and 'pre-exposed') in situ in 6 locations nearby Stockholm, Sweden (3 harbors and 3 references). We hypothesized that snails originating from a clean habitat will have higher mortality rates than those from the pre-exposed population (which is likely to be adapted to contaminants). After 2, 4 and 8 weeks of exposure, we assessed snail mortality and histopathological alterations in all organs (e.g. gonadal atrophy, gill inflammation, parasite infestation etc). As expected, the mortality was significantly higher amongst snails exposed in all harbors, compared to the reference sites. However, the snails originating from the pre-exposed population had higher mortality rates compared to the clean population and this contradicts our hypothesis on adaptation to contaminants. Interestingly, the rate of parasite infestations varied with snail sex differently for the two snail populations, i.e. females had the highest infestation rate for snails from the pre-exposed population, whereas in the clean population males were affected by parasites to a higher degree than females. Analysis of the mitochondrial genome (cytochrome c oxidase I gene) was assessed to determine differences in genetic diversity within and between populations, which might explain differences in tolerance to contaminants.

WP215 Assessing the risk to pelagic species of the cyclic volatile methyl siloxane, Octamethylcyclotetrasiloxane (D4)

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Assessing ecological risks of the widely used cyclic volatile methyl siloxane octamethylcyclotetrasiloxane (D4) to aquatic systems is difficult due to its high volatility and low water solubility. The latter may create environmental labeling issues even when chronic and acute no-observed effect concentrations (NOECs) approximate functional water solubility due to low measured concentrations. This work explores the difficulties inherent in determining the toxicity of D4 to pelagic species and then applies the techniques of probabilistic risk assessment (PRA) and chemical activity to explain the apparent lack of toxicity of D4 to pelagic species under field conditions. Field water samples have been collected and measured for D4 from freshwater and marine sites in Tokyo Bay, many Scandinavian countries, and receiving water downstream of municipal and industrial wastewater treatment plants in Canada; the distribution of measured water concentrations may be compared to the chronic D4 pelagic NOEC values using PRA. In addition, field data on D4 in water and biota may be compared to the activity (fugacity) of the chemical in a variety of media. Chemical activities are simply the ratio of a concentration and its matrix solubility, adjusted for salinity, amount of particulate matter, and carbon content. Activities of concentrations in biota are the ratio of the lipid-based concentration and the apparent solubility of the chemical in lipid, which is approximated by the compound's octanol-water coefficient (K_{ow}) and its aqueous solubility value. This allows expression of all data in a range from 0 to 1, resulting in easy comparison of biota and environmental matrices. The chronic NOEC values for pelagic organisms exposed to D4 were calculated as the aqueous concentration divided by the functional solubility for each test resulting in activities of 0.02 to 1.0, with a median value of 0.53. Based on tissue concentrations in field biota, chemical activities for fish and invertebrates are approximately $1x10^{-7}$ to $1x10^{-6}$, respectively, far less than the NOEC values. Overall, these data show that chemical activities of D4 in biota are not able to reach values that are associated with nonpolar narcosis (i.e., toxicity) and that discharge levels for the past ~30 years have produced field measured concentrations that pose negligible risk to pelagic organisms.

WP216 Complex interactions among agrochemicals in aquatic systems: Can phosphate protect against fungicide toxicity?

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There is a growing concern that Maryland waters can be polluted by surface-water runoff that contains both pesticides and fertilizers. While many pesticides can be toxic to aquatic organisms, less is known about the ecological impacts of phosphate, especially in conjunction with pesticides. Because phosphate is bio-active, such as in the production of ATP, it is possible that phosphate could protect against pesticide toxicity. Previous studies with Daphnia magna have indicated a protective effect of 5.0 mg/L phosphate with regards to survival. Individuals in high treatments of pyraclostrobin (16 µg /l) without phosphate had a survival rate of 20% while individuals with the same concentration of pyraclostrobin and phosphate exhibited a survival of 80%. Additionally there was a trend with regards to time to first reproduction in both the 16 µg/l without phosphate, and 16 µg/l with phosphate treatments, which showed a delayed time to first reproduction compared to the controls. Our next objective was to identify specific concentrations of phosphate that confer a protective effect. We exposed D. magna to a range of environmentally relevant phosphate (KH_2PO_4) and pyraclostrobin concentrations for 21 days. Survival, reproductive output, and size of D. magna were observed. Our working hypothesis is that concentrations of phosphate near 2.5 mg/L will show a protective effect. Importantly, traditional single taxa toxicity tests appear to provide data relevant to limited or unrealistic environmental scenarios and complex stressor designs may provide more ecologically relevant insight. Data like what is reported here may provide information to improve predictive capabilities of environmental managers in ecosystems impacted by complex stressor mixtures of high nutrient and pesticide loads.

WP217 Effects of 2,2′,4,4′-tetrabromodiphenyl ether (BDE-47) dietary exposure on two generations of the marine gastropod Crepidula onyx

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Polybrominated diphenyl ethers (PBDEs) are widely used brominated flame-retardants that have been accumulated in high concentrations in marine sediment and biota. While the biological effects including endocrine disruption and neurotoxicity of PBDEs on mammals and fish are well-documented, information on their effects on invertebrates is scarce. This study aimed at exploring the effects of long-term dietary exposure and parental exposure to environmental level of BDE-47 on marine invertebrate using Crepidula onyx (Gastropod, Calyptraidae). The bioaccumulation and physiological effects on the first (F_0) and second (F_1) generations of C. onyx fed by microalgae encapsulated with BDE-47 were investigated. Transcriptomic analysis by RNA sequencing was also performed to unravel the underlying mechanism. Bioaccumulation was evident in C. onyx from larval stage to adult. Reduction of juvenile survivorship and delay of time to emerge as male in both treatment groups showed physiological impairments of C. onyx upon BDE-47 exposure despite that shell growth and larval settlement percentage were unaffected. Further, reproductive impairment of F_0 adults was clearly evident by the reduction in number of oviposition per female and proportion of well-developed F_1 larvae. The maternally transferred BDE-47 in F_1 offspring had embryotoxic effect as demonstrated by the higher number of broods with non-viable F_1 embryos which failed to develop normally through early cleavage stages (blastula to gastrula). Lastly, the transcriptomic analysis provided potential mechanistic explanations on some of the phenotypic changes observed above, and suggested dysregulation of steroid hormones and alteration of proteins important for embryonic development. These findings have highlighted the need for long-term exposure study which involves multiple generations in toxicological evaluation.

WP218 Export Crops and MRLs: Implications for Ecological Health in Low-Regulation Environments

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Many economically important fruit crops are grown in tropical environments in regions where there are relatively few or lax environmental regulations when compared to North America or Europe. However, these crops are required to meet maximum residue limits (MRLs) in order to be exported and sold in these regions. Because of this, MRLs often constitute de facto regulations in the growing environment that are set by the importing countries. Although this has led to the phase-out of a number of persistent and toxic pesticides in these tropical regions, it has also led to use of less persistent pesticides that nevertheless have very high aquatic toxicity. Therefore, even when fruits like bananas and pineapples consistently meet export requirements, the local growing environment may experience short-term peaks in aquatic concentrations of pesticides following runoff events that result in large fish kills. Here, we discuss dynamic modeling approaches to understanding the time- and application-dependent environmental pesticide levels that can result given the MRLs for current-use pesticides in fruit crops. We present the particular example of banana cultivation in Costa Rica and show how different application patterns, pesticide physicochemical properties and local environmental conditions interact to determine the ecological implications of MRLs defined solely to protect human health.

WP219 GSA-QHTS screening method, a robust and assumption free method to identify hidden drivers of combined stressor effects

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What is the expected combined effect of a set of stressors? When stressors are combined, what is their relative importance? Should we consider all possible stressors for environmental risk assessment? These are some pervasive questions relevant for the understanding and managing of the consequences of chemical pollution in the real world. Modelers recognize that it is difficult to model a highly dimentional system with all its complexity, its interrelations and feedbacks; and that models are most effective when experimental scientists can characterize the main processess and factors driving the system. Experimental scientists are often limited by the experimental costs and uncertainties of laboratory work. This is specially true when trying to understand the combined effect of multiple stressors. In fact, current experimental methods, being esentially reductioninst, are not very well suited to characterize together more than a few number of experimental factors/conditions. In this talk, a novel experimental and statistical integrated analysis framework (GSA-QHTS) is presented. This framework combines Global Sensitivity Analysis (GSA) with Quantitative High-Throughput Screening (QHTS) as a cost-effective integrated framework that allows to screen main drivers of an experimental system response when evaluated under a combined high-dimensional set of candidate explanatory drivers. This set of candidate drivers may include any chemical, physical or biotic factor/stressor that can be set up and control under laboratory experiments. Thanks to GSA parsimony, the experimental cost of GSA-QHTS increased only arithmetically with the number of factors, which is a key advantage when analyzing the relative importance of variables in high-dimensional systems. We will present a case study where GSA-QHTS allowed for the identification of the main pharmaceutical pollutants driving unexpected low-dose mixture effects at the microbial population level in sets of environmentally realistic complex mixtures of pharmaceutical pollutants. In addition, we show how these pollutants were consistently important at the microbial community level in mesocosm experiments. GSA-QHTS has a broader potential adoption across disciplines to identify main effect drivers in high-dimensional sets of candidate factors.

WP220 Incorporating effects of methylmercury on larval fish learning: Scaling up to population dynamics

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Methylmercury has been shown to negatively affect swimming speed and foraging success in larval fishes. These nonlethal effects on fishes can translate into reduced survival due to increased risk of predation and decreased growth rates in young fishes. In addition to impairments to swimming speed and foraging success, larval fishes exposed to methylmercury also exhibit learning deficiencies, including the inability to learn the best prey items to attack, handle and consume. We developed a new learning algorithm that we incorporated into an existing individual-based model (IBM) to determine how impacts of methylmercury on larval fish learning would impact larval growth rates and survival. The IBM followed a cohort of larval yellow perch from hatch through the first 100 days of life. Each day, individual larvae forage, grow, and experience predation and starvation mortality. Methylmercury-induced impairments on learning measured in laboratory behavioral assays were incorporated into the foraging subroutine via changes in handling time and capture success.

We hypothesized that effects on larval fish learning, through reductions in foraging success, would result in significantly slower growth rates and reduced survival than in scenarios in which learning was not affected. Further, these MeHg-induced effects would result in lower survival than effects of MeHg-induced reductions in swimming speed alone. Model results highlight the importance of considering nonlethal effects of toxicants on fish behavior and learning and how these can affect population dynamics. In the future, this model can be used to address questions related to other toxicants and/or species and can incorporate other behaviors affected by toxicants.

WP221 Interaction Effects Across Time and Concentration Gradients in Ceriodaphnia dubia Exposed to Pesticide-Predator Cue Mixtures

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There is a widely recognized need to include greater environmental realism in toxicity studies by considering chemical mixtures and the effects of modifying factors. Predator cues are one biotic factor present in the aquatic environment which, due to the energetically costly responses which they elicit in prey, could produce non-additive effects when combined with pesticides. Another deficiency in standard toxicity testing is the de-emphasis of time as a factor in toxicity. This study addresses the two aforementioned issues — mixtures and toxicity in time — by investigating the temporal dynamics of mixture toxicity. Just as mortality is a function of both concentration and time, interaction effects of mixtures may also be concentration- and time-dependent. In previous studies, Ceriodaphnia dubia were exposed to eight different pesticides separately, either with or without the addition of fish predator cues. In the original analysis, only survival at the 96-h time point was analyzed to test for interactions. The current study, however, utilized data collected at 9-13 time points throughout the exposures in order to characterize the occurrence of interactions across concentration and time gradients. Analysis consisted of fitting parametric distributions to right-censored survival data for cue and non-cue treatments at each concentration over successively longer intervals of time. For example, survival data over the 0-24 h period was first considered, followed by analysis of the 0-30 h, 0-48 h, 0-54 h, . . . , and 0-96 h datasets. Graphical summary of the results depicts a concentration-time “area” in which interactions were detected. In order to compare this area among the eight pesticides, standardized plots were constructed with concentration expressed as toxic units (TUs). Some observed patterns were that interactions generally occurred below ~3 TUs, and as early as 24 h or as late as 96 h. The results of the study provide insight into how single-chemical exposure studies may under or overestimate pesticide toxicity in aquatic habitats due to the presence of environmental factors such as predator cues. The results are also relevant to the design of mixture studies, since the detection of an interaction is highly dependent on the concentrations and duration selected for a study. In addition, the study may be useful in distinguishing mixture effects under exposures of varying duration.

WP222 Methods for Building a Quantitative Adverse Outcome Pathway for Acetylcholinesterase Inhibitors using Bayesian Networks

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Adverse outcome pathways (AOPs) are conceptual constructs that synthesize existing knowledge on the linkages between molecular initiating events and adverse outcomes at higher levels of biological organization. However, AOPs do not currently make quantitative predictions about population scale effects that would be relevant to decision-making. For this project, we are developing a case study that demonstrates how existing toxicological data for acetylcholinesterase inhibitors can be integrated into a quantitative framework that links environmental concentrations to molecular initiating events to population level endpoints using Bayesian network modeling and age structured population models. This poster will present details of the methods used to design the conceptual model, build quantitative relationships between variables, and determine site specific inputs to the model. The case study site is the Puget Sound in Washington, and the endpoint is ESA-listed salmonids including chinook and coho salmon. The model will be designed to predict effects of organophosphate pesticides both individually and in mixture.

WP223 The inclusion of toxic exposures in a population model of Chinook salmon (Oncorhynchus tshawytscha)

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Salmon are a keystone species with a biological foundation that spans from coastal ecosystem health to human economies. Water pollution is becoming an increasingly important salmon conservation issue, particularly in watersheds affected by toxic runoff from urban development, industrial activities, and impervious surfaces such as roads. The physiological effects of many toxic substances are well studied, however the population-level effects are more challenging to evaluate due to differences in life history strategies. Toxic exposures are often not included in population abundance evaluations, particularly sub-lethal exposures or effects on prey abundance. The purpose of this study is to quantify the impacts of toxic insults, such as mortality, sub-lethal changes in growth, behavior, and reproduction, and effects of prey abundance, on Chinook salmon (Oncorhynchus tshawytscha) in the Willamette River basin resulting from exposures to contaminants in Portland Harbor. This study will build upon an existing population model of McKenzie River spring Chinook developed at the NWFSC, representing a sub-basin of the Willamette River. Known tissue residue concentrations from previous sampling efforts of juvenile Chinook at multiple sites throughout Portland Harbor will guide the assignment of chemical exposure. Toxicity will be determined by mechanism of action, and both mortality and sub-lethal effects dose-response relationships. Model scenarios will investigate the impact of toxic insults during smoltification and outmigration on overall population abundance and growth rate. Extrapolations of this model may be applied to Chinook salmon in other watersheds of the Willamette basin and used to guide recovery efforts. Lessons learned from this study will inform similar projects in the Puget Sound and elsewhere.

WP224 Toxicity evaluations of coastal sediments in Osaka Bay, Japan, using Japanese medaka embryos

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Many kinds of chemicals have been discharged into environments with human activities, carried ocean with rainfall, influx of river water, and directly setting from atmosphere. These are settled on sediments eventually. Despite being habitats of numerous species of aquatic organisms on and in sediments, we have only poor data for toxicities of environmental sediments, because it’s difficult to find the direct toxicities of sediments. We consider that sediments composed of soil and pore water. Therefore, new rearing systems of Japanese medaka (Oryzias latipes) embryos on the sediment without water, in order to evaluate toxicities of sediments. In present study, effects to embryos on coastal sediments, collected from Osaka Bay, Japan, were examined with our new toxicity tests, and compared among each sites. Sediments were collected from 15 sites at Osaka Bay closed to big cities as Osaka, Kobe, Nishinomiya, and Amagasaki cities, sifted with 1 mm mesh, and then dried for 72 hours. Dried sediments were washed with embryo raring mediums (ERMs, =pore water), removed extra ERMs with centrifugation, and set into glass petri dish. Three petri dishes per one site were prepared. Fifteen embryos were embedded in half of them in sediments, and incubated at 24°C for 7 days (=exposures to "sediments"). Both exposure periods to two species of medaka depended on the periods until their hatching. Control group with silica sand was also prepared similar with coastal sediment groups. After exposures in each sediments, all embryos were transferred to each one embryo/well into 48 well plates for the examination of effects such as mortality, hatching rate, growth rate and malformation rate. As a result, sediments collected around river mouth of Yodo River flowing in big city as Osaka city caused relatively high decreases of hatching rates with 60%. In addition, slight decreases of hatching rate (80%) were observed around river mouths of others. These results suggest that rivers flowing into Osaka Bay certain caused sediment toxicities to medaka embryos. Another site close to Osaka city was observed extreme hatching delay (16.2 ± 2.32 dpf) of hatch compared with control (10.4 ± 0.946 dpf). Similar tendencies for hatching delay were observed in sediments collected from coastal area around Osaka to Nishinomiya cities. However, we cannot found extreme effects on sediments collected around Kobe city.

WP225 When is it close enough? An evaluation of complexity trade-offs for modeling pesticide run-off risk with VFSMOD

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A main issue when building models that represent the "real" behavior of a system is the complexity dilemma: how much complexity is required to get a close enough approximation of the system´s behavior? When is it close enough? While these questions may look rather philosophical, they cascade into major practical, economical and regulatory consequences. We will present a frameworks: Global Sensitivity and Uncertainty Analysis (GSA/UA) as pragmatic set of tools to deal with these questions. GSA/UA a global understanding of model sensitivity and uncertainty, and to rank model input factor´s relative importance when all factors varied together. One potential applications of GSA/UA is to guide the reduction of model complexity without losing model explanatory power. We will illustrate the usefulness of both frameworks with a series of case studies focused on the analysis of factor importance determining the efficacy of vegetative filter strips (VFSs) for reducing pesticide run-off risk to the aquatic compartment as represented by the process-based model VFSMOD (Vegetative Filter Strip Modeling System). Since the efficacy of VFSs depends on site-specific input factors, relationships of filter efficiency with filter length and other input factors may result in rather complex and non-linear relationships. We will illustrate how some model parameters assumed to be critical in endless discussions in different regulatory frameworks, may reveal themselves as non-relevant when measured in a common scale metric with all the other input factors present.

Demonstrated Remediation Technologies Addressing Contaminated Soil, Sediment and Water

WP226 Transformation of the Recalcitrant Pharmaceutical Compound Carbamazepine by the White Rot Fungus Pleurotus ostreatus

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Concern over the exposure to active pharmaceutical compounds (PCs) in the environment is increasing. Many PCs are not fully removed during municipal wastewater treatment; thus they are released to the environment with the treated effluents. Consequently, PCs and their potentially toxic degradation products may be found in surface and groundwater and being introduced into agricultural soils, where they can accumulate and be taken up by crops. Although PCs are found in the environment at very low concentrations, this is of health concern since the consequences of chronic exposure to a mixture of various PCs and their degradation products is largely elusive. Carbamazepine (CBZ) is a widely used anticonvulsant drug which serves as a model compound in the study of environmental exposure, implications and removal of PCs. This study aims to elucidate the metabolic pathway of CBZ by P. ostreatus grown in liquid media and on a natural lignocellulosic substrate. When P. ostreatus was grown in liquid medium 99% of the added CBZ were transformed to the 10,11- epoxy CBZ by cytochrome P-450 and Manganese Peroxidase. At the end of the incubation period a new product, 10,11-Dihydro-10,11-dihydroxy CBZ, was formed. Both products showed a limited removal also when they were applied as primary substrates. When P. ostreatus was grown on cotton stalks, 90% of the added CBZ were removed after 60 incubation days. In contrast to the findings in the liquid medium, the removal rate of eoxy-CBZ and dihydroxy-CBZ as primary substrates exceeded 90% and new 22 transformation products were discovered. epoxy-CBZ was further metabolized via acridine and dihydroxy-CBZ pathways. The latter was further metabolized via five sub-pathways. Some of the identified transformation products have been reported to be formed by oxidation of CBZ by abiotic agents, but never as a result of a biological processes. When ^{14}C-carbonyl-labeled CBZ was used as the substrate, ^{14}C-CO_2 release amounted to 17.4% of the initial radioactivity. This release can be attributed to at least two reactions where the carbonyl group was eliminated. However, due to the position of the radioactive labeling it is impossible to tell whether a mineralization has occurred. This work highlights the effect of growth conditions on the degradation pathway of PCs and formation of possibly toxic degradation products. This is of importance in the context of release of PCs to the environment, e.g. irrigation with treated wastewater.

WP227 Remediation efficiency of three treatments on water polluted with endocrine disruptors: Assessment by means of in vitro techniques

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Chemical substances with potential to disrupt endocrine systems have been detected in aquatic environments worldwide, making necessary the investigation about water treatments able to inhibit such potential. The present work aimed to assess the efficiency for removing endocrine disruptors (with estrogenic and androgenic activity) of three simple and inexpensive substrates that could be potentially used in sectors or regions with limited resources: powdered activated carbon (PAC), powdered natural zeolite (ZEO) (both at a concentration of 500 mg.L-1) and natural aquatic humic substances (AHS) (at 30 mg.L-1). MilliQ-water and mature water from fish facilities (aquarium water, AW), were artificially contaminated with 17β-estradiol (E2), 17α-ethinylestradiol (EE2) and dihydrotestosterone (DHT). Moreover, effluent samples from waste water treatment plants (WWTP) were also submitted to the remediation treatments. In order to assess estrogenic and androgenic activities two cell lines permanently transfected with luciferase as reporter gene under the control of hormone receptors were used: AR-EcoScreen containing the human androgen receptor (hAR) and HER-LUC transfected with the sea bass estrogen receptor (sbER). Results showed that only PAC was efficiently removing the estrogenic and androgenic compounds added to milliQ and AW. However, androgenic activity detected in WWTP effluents was only reduced after treatment with ZEO. It seems that hormonal activities detected in WWTP effluents are influenced by the plethora of substances present in them, that probably not only lead to antagonistic, agonistic or additive effect, but can also affect the adsorption of target molecules to the matrices used in this study. Such a possibility must also be explored.

WP228 Assessment of ozonation efficiency to disinfect municipal effluents

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The disinfection of wastewater is a technology recognized for its efficiency in many cities of the world, however if that disinfection is based on oxidation, the composition of the effluent can affect the treatment efficiency. The variation in reactivity and composition of the effluent can delay the inactivation of microorganisms due to the presence of competing reactive material to react with oxidants. For advanced oxidation processes, the radicals formed, mainly HO• may have its efficiency affected by varying the reactivity of organic matter (EFOM – effluent organic matter) and inorganic compounds such as carbonates and nitrates. Currently, the potential of oxidative processes to disinfect effluents is known as well as that the variation in composition and reactivity of the effluent can affect the treatment. However, it is still not clear how each component, components of EFOM, inorganic compounds and even chemical compounds of industrial origin may affect the process. The objective of this work was to verify the efficiency of ozonation to disinfect municipal effluents as well as to assess the toxicity variation along the treatment time. Initial results demonstrate that ozonation was able to remove E. Coli in the first minutes of ozonation, equivalent to a ozone dose less than 10 mg L^{-1}. Preliminary acute toxicity tests demonstrated that ozonation was able to reduce the acute toxicity with the ozone dose necessary to the complete disinfection.

WP229 Catalytic reduction of Cr(VI) and chloroacetic acids using Au@Pd/TiO2 and Pd/TiO2 catalysts

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Cr(VI) is a commonly identified heavy metal pollutant in the aquatic environment. It is listed as one of the priority pollutants by the USEPA due to its high acute toxicity to humans. Cr(VI) can be reduced to a less toxic form, Cr(III), which can be removed subsequently at neutral or alkaline environments through precipitation. Chloroacetic acids (CAAs), one of the disinfection byproducts (DBPs) formed in chlorination for drinking water treatment, attract significant concerns because of their strong carcinogenic, mutagenic and hepatotoxic effects. CAAs could be effectively dechlorinated into nontoxic acetic acid during reduction process. The catalytic reduction is a promising method to remove inorganic pollutant Cr(VI) and organic pollutant CAAs in water. We prepared Au@Pd catalysts supported on TiO_2 with delicate core-shell structures by a two-step photo-deposition method. The catalytic reduction performances of Au@Pd catalysts on Cr(VI) and CAAs are studied and compared to the performance of monometallic Pd catalysts supported on TiO_2. In this study, Au loading amount of bimetallic Au@Pd/TiO_2 catalysts was kept at 0.99 wt.% and Pd loading amounts varied from 0.05 to 0.15 wt.%. Pd loading amount of monometallic Pd/TiO_2 was 0.05 wt.%. The results showed that Pd atoms preferentially deposited on the exposed Au surface to form core-shell structured Au@Pd particles in the preparation of the bimetallic catalysts. The bimetallic catalyst Au@Pd/TiO_2 and the monometallic catalyst Pd/TiO_2 displayed completely different catalytic activities on the reduction of Cr(VI) and CAAs. For Cr(VI), Pd/TiO_2 showed negligible catalytic activity, while Au@Pd/TiO_2 exhibited a remarkable conversion percent of Cr(VI). The catalytic activity of Au@Pd/TiO_2 increased with the increase of Pd deposition amount from 0.05 to 0.10 wt.%, whereas remained the same when Pd deposition increased from 0.10 wt.% to 0.15 wt.%. For CAAs, Pd/TiO_2 exhibited higher activity than Au@Pd/TiO_2 and the reduction efficiency monotonically increased with the increase of Pd loading amount from 0.05 to 0.15 wt.%. In summary, the environmental pollutants Cr(VI) and (CAAs) in water can be converted to a less toxic form by catalytic reduction. The bimetallic catalyst Au@Pd/TiO_2 showed a higher activity in reducing Cr(VI) and the monometallic catalyst Pd/TiO_2 performed better in treating CAAs. The catalytic performance of both the catalysts can be optimized by controlling the loading amount of Pd.

Remediation/Restoration – Poster Only

WP230 4-Chlorophenol Degradation in Aqueous Media, Using Sono-electro-Fenton Method

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Electro Fenton (EF), ultrasound radiation and Sono-electro-Fenton's process (SEF) all are advanced oxidation process (AOP) that have drawn great interests for removal of chloro-organics from water. EF and ultrasound radiation are known to produce hydroxyl radicals that are strong oxidative agents. The main objective of this study was to combine these two processes to degrade 4-chlorophenol (4-CP) using SEF process in an aqueous solution and to investigate the effects of different parameters on contaminant removal efficiency. Initial pH, current, ferrous iron concentration and Pd/Al_2O_3 catalyst dosage were optimized in a two electrode (Ti/mixed metal oxide or Ti/MMO) batch system using electro-Fenton (EF) mechanism. The SEF was then tested with a pulsing 20 KHz ultrasound wave to optimize pulsing frequency and sonifiers amplitude. EF tests were conducted with a 200 mg L^{-1} 4-CP initial concentration and a 10 mM sodium sulfate (Na_2SO_4) as background electrolyte. More than 90% of 4-CP was removed within 300 minutes in the presence of 80 mg L^{-1} Fe(II), 200 mA of current, 1 g L^{-1} Pd/Al_2O_3 catalyst and initial pH of 3. In the SEF process where 70% amplitude was used along with a 5.9 and 59 seconds pulse on and pulse off, respectively, the removal rate of 4-CP degradation increased within the first 120 min compared to EF. However, degradation slowed down over time and complete 4-CP removal was observed after 300 minutes. Complete results along with the energy consumptions will be presented.

WP231 Analysis of amino acids of agricultural interest in composting

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Composting is a sustainable approach to manage animal and vegetal waste generated in the Fundação Parque Zoológico de São Paulo (FPZSP). The resulting compost is often used in ZOO's premises as organic fertilizer for the production of vegetables, which is further used to feed the animals. The composting product provides many forms of mineral and also amino acids (AA) that are absorbed by plants as nutrients. Since most amino acids absorb only slightly or not at all in the UV wavelengths, we developed a method for the determination of AA of agricultural interest in the composting samples. Due to the complexity of samples, we used ion exchange chromatography for purification of AA prior analysis. The proposed CZE-C4D method allowed a separation of the AA in a short analysis time (less than 3.0 min), with great linearity (ranging from 0.993 to 0.998). Using a BGE of 10 mmol L^{-1} TEA, reduction of high-frequency noise and lower baseline fluctuations were obtained. The LOQ for the five AA were around 35 µmol L^{-1}, and were adequate for our purpose. In addition, the method showed good precision (RSD of peak area and migration time less than 1.55 and 1.16%, respectively).

WP232 Avoidance behavior of Eisenia andrei to Achromobacter spanius inoculated mancozeb and copper oxychloride pesticides spiked soils

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The Earthworm avoidance behavior test is a rapid screening tool for the assessment of soil contamination and habitat function. Its short duration and cost effectiveness therefore makes it appropriate for quick risk assessment of pesticides in soils. Mancozeb and copper oxychloride are two commonly employed metal-based fungicides in vineyards and orchards. While mancozeb contains 16 % manganese (Mn) and 2 % zinc (Zn), copper oxychloride is composed of 60 % copper (Cu). In the present study, the avoidance test (ISO 17512-IFDIS, 2007) was conducted to assess the preference behavior of earthworms – Eisenia andrei in bacterial inoculum-pesticides soil substrates. A 24 hours broth culture of bacterial strain – Achromobacter spanius (KT819127) isolated from gold and gemstone mining site (and shown tolerance to 600, 300 and 200 ppm Mn, Zn and Cu respectively in a previous study) was introduced into mancozeb and copper oxychloride OECD spiked soils. The two-chamber design avoidance test was adopted. Five replicates of 10 earthworms (300 – 600 mg) were exposed in control and test substance of mancozeb (8, 44, 800 and 1250 mg/kg) and copper oxychloride (200, 450, 675 and 1000 mg/kg) and incubated at 23 ± 2 ^{0}C for 48 hours. The results revealed that at mancozeb 44 and 800 mg/kg, there was a net response of 8 and 92 % with the inoculum-pesticide test substrate while an avoidance response of 28 and 100 % was recorded in the non-inoculum-pesticide test substrate. Likewise for copper oxychloride 200 and 450 mg/kg, while a 100 % net response was recorded in the non-inoculum-pesticide substrate, an 87.5 and 96 % avoidance behavior was obtained with the Achromobacter spanius inoculum test. These results show some higher preference by Eisenia andrei for inoculum-pesticide substrates compared to the non-inoculum-pesticide substrates. However, a highly reduced habitat function was demonstrated by earthworms at mancozeb 800 and 1250 mg/kg and copper oxychloride 675 and 1000 mg/kg in both substrates with and without bacterial inoculum. The findings of this study therefore indicate that Eisenia andrei showed lower avoidance behavior towards inoculum-pesticide substrates compared to non-inoculum pesticide substrate at reduced mancozeb and copper oxychloride concentrations. Thus, a further study to determine acute and sublethal toxicity effects of mancozeb and copper oxychloride on Eisenia andrei in the presence of Achromobacter spanius will be conducted to confirm these findings.

WP233 Bioefficiency of Rhodanese Produced by Klebsiella edwardsii in the Clean-up of Cyanide Polluted Water

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Water bodies used for cassava processing contains cyanide which is toxic to several forms of life. The use of enzyme for the remediation of polluted environment presents advantages over traditional technologies and also over microbial remediation. Extracellular rhodanese of strain of Klebsiella edwardsii isolated from cyanide polluted stream which is used for cassava processing was studied. Biochemical characteristics of the purified enzyme, including pH, temperature profiles, kinetic parameters, substrate specificity and effect of metallic salts were also determined. Production of rhodanese correlated with the rate of bacterial growth at the death phase and the optimum incubation time for maximum enzyme production was 48 h. The enzyme was purified by 85 % ammonium sulphate precipitation and DEAE-Cellulose ion-exchange chromatography. The purified enzyme was introduced to the cyanide-polluted water varying the conditions to assess the remediating ability. The pure enzyme had a specific activity of 0.0473 mg^{-1} with a purification fold of 4.56 and a percentage yield of 30.30 %. The Michaelis-Menten constants (Km) values of rhodanese from Klebsiella edwardsii for potassium cyanide and sodium thiosulphate were 50 mM and 200 mM respectively while their maximum reaction velocities (Vmax) were 0.56 RU/ml/min and 1 RU/ml/min respectively. The substrate specificity study showed the percentage utilization of the various substrates to be: 2-mercaptoethanol (33.7 %), ammonium persulphate (122.9 %), ammonium sulphate (178.3 %), sodium metabisulphite (67.5 %) and sodium sulphite (78.3 %). The enzyme demonstrated a broad pH range but the optimum pH was at 6.0 while the optimum temperature was 60 °C. The inhibition study on the enzyme by salts ($HgCl_2$, $MnCl_2$, $NiCl_2$ and $ZnCl_2$ and KCl) at concentrations 1 mM, 10 mM, 50 mM and 100 mM showed that the enzyme was inhibited by all the salts except for $HgCl_2$ at concentrations 50 mM and 100 mM. The results showed that the presence of rhodanese in the Klebsiella edwardsii suggests that the enzyme may possess functional cyanide detoxification mechanism necessary for the survival of the organism in the environment. Also, highest activity of rhodanese was achieved under optimum temperature and pH as well as using the polluted water as both cyanide and sulphate sources. The study therefore concludes that rhodanese can be used in a cheap and environmentally way to clean-up cyanide polluted water.

WP234 Can we improve extremely HM contaminated soil as habitat for E. fetida, using wood ash, biochar or humic substances?

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Heavy metals are considered priority pollutants of environmental concern. But contrary to organic pollutants, HM cannot be degraded and thus constitute a persistent environmental hazard. We focus on chemical and ecotoxicology influence of Wood ash (WA), Biochar (BC, derived by pyrolysis of willows and poplars at 600C) and Humic substances – Lignohumate-K (LG) into fluviosoil. Native soil (NA) contained Zn 521±21 ppm, Cd 127.4±3.6 ppm, Cu 5.7±1.6 ppm, Pb 3035±26 ppm. A greenhouse pot experiment was carried out for 60 days. Soils were treated with different types and levels of amendments (2.8, 6 and 8.4% for WA; 0.05, 0.5 and 5% for BC; 0.05, 0.5 and 1% for LG) according to the final soil pH(CaCl2) value: 6.0, 6.5 and 7.0. Results revealed that Cd, Cu, Pb and Zn concentrations were significantly reduced in amended soils. WA showed the highest ability to immobilize HM. We observe the mobility decrease of 35% for Cd, 70% for Cu and 75% for Pb with no significant difference between amendment levels. For Zn 2.8%WA significantly reduced mobility to 47.49±4.18%, 6% WA to 55.13±3.00% and 8.4% WA to 69,12±7.44%. BC had no difference between levels immobilizing about 35% Cd, 65% Cu, 75% Pb, 50% Zn. LG showed the same results, but we point out that the lowest level of LG was the most efficient. NA soil showed the toxic effect on the E.fetida. Higher survival rate in amended with LG and WA soil indicated that remediation measures improved soil quality. BC treatments had a detrimental effect in soil toxicity, induced earthworms mortality, up to 100% after first 3 days. We also noted weight changing of earthworms body after 60 days. In NA and LG treated soil individuals had weightloss of 25-50% and 40-90%, while WA treatment leaded to 25% weight increase. Also, we estimated bioavailability of HM for E.fetida in contaminated soil with amendments of WA and LG. WA in 2.8% and 8.4% level reduced tissue concentration of Cu, Zn and Pb of about 40%. For 6% WA and all variants of LG no significant influence was observed. The results showed that mobility of HM can be reduced by all three amendments. But, BC and LG had negative ecotoxicological influence in the form of increased mortality and weight loss of E.fetida. We suggest that determination of the pollutants content is not enough to fully evaluate soil quality, because the ecotoxicological danger in the environment is not reflected by these factors. The research was supported by the RFBR project No.16-34-60011 mol_a_doc.

WP235 CO2 flux from oil contaminated intertidal sediments to evaluate in-situ biodegradation of oil: a mesocosm study

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CO_2 fluxes were measured to monitor biodegradation of oil in mesocosms of oil contaminated sediments for 71 days. Mesocosms, placed in an intertidal zone exposed to semidiurnal tides, were constructed by mixing a given amount of crude oil with sediment from the site. Experimental groups include control (native microbes; NM), NM + fertilizer (F), NM + a commercial biological enzyme (ENZ), NM + a commercial nutrient based stimulant (STM), NM + activated carbon, F + effective microorganisms (EM), and F + a consortium of oil degrading microbes (ODM). Change of CO_2 concentrations in a closed chamber system was measured using an infrared gas analyzer to calculate CO_2 flux from the artificially contaminated sediments in each experimental group. On average, CO_2 evolution was highest in the F + ODM group (0.89 g CO_2 m^{-2} hr^{-1}), followed by NM + ENZ group (0.45 g CO_2 m^{-2} hr^{-1}) and F + EM group (0.38 g CO_2 m^{-2} hr^{-1}). Changes of CO_2 evolution were not obvious in the other groups. The highest flux was noticed at 4 days after the treatment in a NM + ENZ group (1.50 g CO_2 m^{-2} hr^{-1}) which was about six times higher than those from a control group (0.24 g CO_2 m^{-2} hr^{-1}). Thereafter, the CO_2 flux was decreased drastically and return to the level of control in 15 days after the treatment. In contrast, CO_2 evolution from the F + ODM group was maintained relatively high during the whole experimental period, with a maximum CO_2 evolution at day 23 (1.43 g CO_2 m^{-2} hr^{-1}). Maximum evolution in EM treatment was also observed at day 23. Initial effect was higher for the enzyme treatment but it diminishes quickly while the effects of microbe inoculations (ODM and EM) last relatively longer. The effects of the treatments were also confirmed by monitoring the changes of the concentration and composition of oil components in the sediments.

WP236 Competitive Adsorption of Reactive Black 5 Dye onto Sawdust of Parkia biglobosa

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The development and modification of existing processes for the abatement of pollutants in the environment is a continuous exercise. In this regard, the importance of wastewater treatment before its eventual discharge into the environment cannot be overemphasized. Adsorption process has been widely accepted as an efficient method, and research is ongoing on the possible application of agricultural residues as adsorbents in wastewater treatment. This study investigates the potential of Parkia biglobosa sawdust in removing Reactive Black 5 dye from aqueous solutions in single and binary dye systems. Several works have been conducted on the adsorption of reactive dyes but reports on their adsorption as a component of a mixture of dyes is scanty. The sawdust, collected from a local sawmill, was thoroughly washed, dried and characterized using Fourier Transform-Infrared (FT-IR) Spectrophotometer and Scanning Electron Microscope (SEM). Batch adsorption experiments were conducted to determine the effects of adsorbent dose, initial concentration and pH of the dye solution. Five isotherm models were employed in the interpretation of the equilibrium experiments. Kinetics and thermodynamics of the adsorption processes were also studied. The results indicate the presence of functional groups which could be potential adsorption sites for interaction with the dye on the sawdust. The kinetics of the adsorption in both single and binary systems could best be described by the Pseudo-second order model; and Langmuir gave the best fit for the equilibrium adsorption data ($R^2 > 0.94$). The maximum monolayer adsorption capacity of the adsorbent for the dye was low (2.21 mg/g) with no significant difference in the two systems. The adsorption processes in all the systems were spontaneous with DG becoming increasingly more negative (-0.15 to -2.81 kJ/mol) as temperature increases. The enthalpy change (ΔH) for the sorption in both systems was positive, indicating that the process was endothermic; the change in entropy (ΔS) was also positive an indication of increased disorderliness on the surface of the adsorbent.

WP237 Laser photo-stimulation of plants and microbes associated with plants, for better phytoremediation of heavy metal contaminated sites

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Empirically selected wavelengths and exposure times of low level lasers has resulted in immense possibilities of using plants for reclamation of degraded land masses and clean-up of contaminated water resources. Willow (Salix viminalis), which is one of the hyper-accumulator plants, used in the experiment, showed a remarkable increase in shooting and rooting, when the cuttings cultivated in hydroponic condition were photo-stimulated with low power lasers. Photo-stimulation with red beam Helium-Neon laser (power=20mW, wavelength= 660 nm) for 3 times at 10 seconds intervals after each 10 seconds irradiation followed by 3 times irradiation with blue beam Laser Diode (power=25mW, wavelength=514nm) for 3 seconds with 3 seconds pause after each irradiation was highly effective in increasing root and shoot growth. The increase in growth of plant parts, after laser irradiation, is attributed to an increase in the rate of adenosine triphosphate (ATP) production. The enhanced rate of energy production in plants and microbes associated with plants make them able to adapt and clean up the sites that are highly contaminated with heavy metals and environmental pollutants.

WP238 Metagenomic study of polystyrene degradation by meal worms

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In the last 50 years, there has been a rapid increase in plastic usage in almost every sphere of life. Polystyrene (PS) or Styrofoam, as it's more commonly known, has been used in various products like cups and packing material and also as a raw material and solvent in the processing of polymers. Its uncontained production, constant daily usage and incorrect disposal have led to grave environmental problems including destruction of wildlife habitats, and bioaccumulation in food chains. Until very recently, it was assumed that polystyrene was non-biodegradable. A study conducted in 2015 showed the ability of meal worms to degrade polystyrene; the ability was attributed to their gut microbiome (Yang et al., 2015). The researchers concluded that the degradation must occur due to the secretion of enzymes by the gut micro-organisms. A metagenomic study focused on PS degradation and the gut microbiota has not been carried out to date. Mealworms, at different instars, were subjected to a diet of only Styrofoam for a period of 2 weeks and subsequently dissected to obtain intact guts. In order to determine the genomic content of the gut, rRNA was extracted, converted to cDNA and processed to acquire the 16S metagenome (for diversity and abundances). The bacterial v3 and v4 variable regions were used as primer targets during the sequencing. In this study we report on the metagenomic diversity and identification of the major as well as active species involved in the gut microbiota of mealworms with Styrofoam as their only food source compared to mealworms fed a normal diet. A change in diet reflects change in abundances of the microbial community (Ley et al., 2008) and the same was expected when the mealworm diet shifted from bran and oats to Styrofoam. Results from this study contribute to understanding of the functionality of this mixed community that enables it to degrade PS successfully. Applications for landfill management as well as plastic recycling, one of the most intractable problems of human society today are expected deliverables from this study.

WP239 Modeling reactive species transport within the electrochemical remediation process in a laboratory-scale flow-through reactor

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Groundwater electroremediation involves applying direct current across electrodes immersed in groundwater to intercept and transform contaminants through direct and/or indirect oxidation and reduction reactions. The system undergoes significant changes in pH due to water electrolysis. Considering that pH is one of the most important parameters which controls reactions and influences the transformation mechanisms, a theoretical model can be a sufficient tool for understanding the change of pH during the process and adjusting the system for a full-scale implementation. However, developing new models is challenging due to multicomponent species transport. Here we present a theoretical model to describe pH changes during electrochemical processes with the use of inert (mixed metal oxide, MMO) anodes. Experimental data has shown that, pH within the system can alter within a wide range of 2 to 12. We developed the theoretical model which includes chemical reactions, water auto-ionization, and electrolysis to describe the dynamic changes in chemistry across the cell. The model accounts for direct electrolysis reactions of the species. The model study is considering different current reversal ratios and frequencies as well as different electrolyte concentration and composition. The model's output is compared with experimental studies where it showed a god correlation (R^2=0.7) between the theoretical results and observed data.

WP240 Morphological, physiological, biochemical and yield responses of lady finger grown on different MSW vermicompost amendment ratio

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Using municipal solid waste (MSW) vermicompost is one of the most promising and an alternative option for management of solid waste. A pot experiment was conducted to study the morphological, physiological, biochemical and yield responses of lady finger (Abelmoschus esculentus L.). MSW vermicompost (VC) was applied in four different amendment ratios (VC: Soil) viz. A (4:1), B (4:2), C (4:3) and D (4:4). Apart from that, plants were grown in soil as control and also in recommended dose of inorganic fertilizer (IF) for comparison. Total chlorophyll, carotenoid and protein content were reported to increase in all the amendments including 'IF' except 'A' as compared to control. Similarly, lipid peroxidation, peroxidase activity and proline content were also reported to increase significantly, particularly in A and B, however, thiol and phenol content decreased as compared to control in plants grown in different amendments. Photosynthetic rate (Ps) and stomatal conductance rate increased in different treatments except 'A' as compared to control. Likewise, total leaf area, specific leaf area, leaf area ratio, leaf weight ratio and total biomass increased in plants grown in different amendment ratios of MSW vermicompost except 'A' in comparison to control. In addition to this yield was also reported to increase in all the treatments except 'A' and 'B' with maximum increase in D (136.8%), which was followed by C (82.92%) and IF (38.59%). However, a decrease of 66.32% was noticed in 'A' which was followed by B (3.57%). The study concluded that MSW vermicompost may be good option for lady finger as plants has adequate tolerance mechanism showed by increased rate of photosynthesis and chlorophyll content, various antioxidant levels and yield (B, C & D). However, at higher dose of MSW vermicompost (A), plants growth decreased therefore, this dose is not recommendable for application of MSW vermicompo st in lady finger.

WP241 Performance of commercially available soil amendments for enhanced copper removal in bioretention media

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Bioretention structures such as planter boxes, swales and rain gardens are being increasingly utilized in built landscapes as a strategy to attenuate both stormwater flows and contaminant loads. Copper roofing materials can contribute significantly higher mass loads of dissolved copper per unit area than other surfaces such as parking lots and roadways. While a recent study demonstrated that conventional bioretention media can remove over 90% of copper from copper roof runoff, the median discharged Cu concentrations from bioretention structures (66 μg L^{-1}) still did not achieve the lowest copper levels sought by some jurisdictions (< 14 μg L^{-1}). In this laboratory column study, three commercially available soil amendments, sold as fertilizers and soil conditioners, were evaluated for their ability to enhance the performance of bioretention media for copper removal. Biochar, greensand, and zeolite were tested; each of the amendments was prepared in both a layered and mixed treatment. Artificial stormwater containing 2000 μg L^{-1}Cu was added, to each column in 2.00 L increments using a peristaltic pump at 10 cm/hr flow rate to simulate natural rainfall. This pilot study demonstrated that the addition of these amendments generally promoted enhanced copper removal compared to the controls; however, greensand and zeolite tended to be more effective than biochar and layered treatments tended to be more effective than admixtures for greensand and biochar. The copper exports from the control ranged from 34-84 μg L^{-1} and amended columns typically exporting copper concentrations around 50% of the control. Extended duration column studies are ongoing for each of the soil amendments as both admixtures and treatments at varying ratios. Artificial stormwater containing 4000 μg L^{-1} Cu is being added in 2.00 L increments using a peristaltic pump at a 10 cm hr^{-1} flow rate to simulate maximum concentration and event volume conditions exhibited in a previous field study of runoff from copper roofing material. These studies utilizing biochar and greensand amendments have shown promising results. The biochar layered amendments consistently performed better than the control columns at both volume ratios and consistently achieved effluent concentrations below the 14 μg L^{-1} threshold. The greensand amendments were less consistent than biochar columns, with respect to performance compared to control; however, both greensand mixed amendments consistently achieved effluent concentration below the 14 μg L^{-1} threshold. The column study for zeolite is currently ongoing. In addition, cation exchange capacity is being determined for each treatment. Further studies will evaluate the most effective amendment, treatment, and volume configuration in a field study to determine effectiveness when subjected to runoff from copper roofing material.

WP242 Performance of Hybrid Pilot-scale Constructed Wetland Systems for Treating Oil Sands Process Affected Water from the Athabasca Oil Sands Area

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Mining leases in the Athabasca oil sands area (AOSA; near Ft. McMurray, Canada) produce large volumes of oil sands process-affected water (OSPW) that contain potentially problematic constituents requiring treatment prior to surface water discharge into receiving aquatic systems. The aim of this research was to identify constituents of concern (COCs) in OSPW from an AOSA tailings pond and design hybrid pilot-scale constructed wetland treatment systems (CWTS) to mitigate risk to a sentinel aquatic species. COCs were identified based on comparisons to ambient water quality criteria for the protection of aquatic life (i.e. Canadian Environmental Quality Guidelines [CEQGs], Alberta Environment Water Quality Guidelines [WQGs], and United States Environmental Protection

Agency [USPEA] water quality criteria) and toxicity endpoints. Specific COCs identified in the OSPW include organics (naphthenic acids [NAs], oil and grease [O/G]), metals/ metalloids (Al, As, Cu, Ni, and Zn), and suspended solids. Characteristics of the OSPW are NA concentrations from 80 to 128 mg/L ("total" NAs quantified by HPLC), slightly alkaline, pH 7.91 to 8.45, and concentrations of metals/ metalloids exceeding numeric guidelines for protection of aquatic life. The hybrid pilot-scale CWTS were designed to promote treatment processes to alter (transfer and transform) the COCs using sequential reducing and oxidizing wetland reactors and a solar photocatalytic treatment reactor using fixed film titanium dioxide (TiO_2). Performance assessment of the hybrid pilot-scale CWTS used multiple lines of evidence, including rate and extent of COC removal and measured toxicity using the aquatic invertebrate Ceriodaphnia dubia. The CWTS decreased concentrations of NAs, O/G, and metals below performance criteria and decreased toxicity to C. dubia. Results from this study provide proof-of-concept data to inform hybrid passive or semi-passive treatment approaches (i.e. constructed wetlands) that could be used to mitigate COCs contained in OSPWs.

WP243 Performing Ecological Evaluations for Sites Under a Privatized State Remediation Program

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The privatization of contaminated site cleanup is a relatively new paradigm to streamline the investigation and remediation of an often overwhelming number of contaminated sites. Since Massachusetts initiated the first Licensed Site Professional (LSP) program in 1993, several other states have implemented similar programs, including New Jersey, with the Licensed Site Remediation Professional (LSRP) program, and Connecticut, with the Licensed Environmental Professional (LEP) program. Although the various programs privatize different aspects of environmental cleanup and some place significant restrictions on the types of sites eligible for management within each program, the overall goal of privatization is to reduce the contaminated site backlog within states and provide a cost effective approach to site closure. Site remediation programs typically require remedial approaches and remedies that are protective of both human health and the environment, which includes ecological resources and sensitive receptors. Under these programs, licensed professionals are obligated to reach conclusions regarding the protectiveness of remedies relative to both human health and ecological impact, and often without direct State involvement in decision-making. However, regulatory programs governing human health protection are typically well structured and mature, while the protocols for investigating potential impacts to ecological receptors are often less so. For example, while ecological screening criteria are generally available for surface water, sediment, and surface soil, few formal state-specific protocols exist to identify ecologically sensitive receptors, determine complete ecological exposure pathways, develop and use conceptual site models, and evaluate the availability of chemicals of interest to ecological receptors. Without these protocols, licensed professionals must rely on other agency or technical guidance, such as USEPA or NOAA, to develop testing, evaluation, and risk assessment approaches for ecological resources. These decisions require judgements that can result in inconsistently applied remedies. This presentation explores how ecological investigations are implemented under privatized remediation programs and highlights their strengths and weaknesses.

WP244 Polymer/biomass-derived biochar for use as a sorbent and electron transfer mediator in environmental remediation

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Co-pyrolysis of polymer and biomass wastes was investigated as a novel method for waste treatment and synthesis of enhanced biochar. Co-pyrolysis of rice straw (RS) with polypropylene (PP), polyethylene (PE) or polystyrene (PS) increased the carbon content, cation exchange capacity (CEC), surface area and pH of the biochar. As a result, the sorption of 2,4-dinitrotoluene (DNT) and Pb to polymer/RS-derived biochar was markedly enhanced. The increased aromaticity and hydrophobicity may be responsible for enhancing the DNT sorption to the polymer/RS-derived biochar. In contrast, increasing CEC, higher pH, and the newly developed surface area may account for the enhancement in Pb sorption. The addition of polymer to RS did not significantly change the catalytic role of biochar during the reduction of DNT by dithiothreitol. Our results suggest that co-pyrolysis of RS and polymer can improve the biochar properties to enhance the sorption of DNT and Pb.

WP245 Redox transformation of explosives in contaminated soils using Fe-bearing materials: Kinetics and toxicity of treated soils

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The oxidative and reductive transformation of nitro explosives in contaminated soils was examined via batch experiments conducted with Fe-bearing materials. Zero-valent cast iron (Fe(0)), steel dust from a steel manufacturing plant, and FeS can rapidly reduce 2,4,6-trinitrotoluene (TNT) and hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) in soil under anaerobic conditions as long as a sufficient amount of water is present. Fe-bearing materials can also effectively activate persulfate ($S_2O_8^{2-}$) so as to enhance the oxidative transformation of TNT and RDX in soil-water systems. Kinetically, reductive and oxidative transformations led to the removal of more than 90% of the explosives from a soil-water system within 5 h under the given conditions. Pseudo-first-order rates in the range of 0.7 – 23.4 h^{-1} were observed. By increasing the concentration of persulfate or Fe-bearing materials, the oxidative transformation could be promoted. Treated soils via redox reactions using Fe-bearing materials did not show significant toxicity, except for the case of TNT-contaminated soils oxidized by FeS-activated persulfate. Considering the kinetics of explosive degradation and the toxicity of treated solutions and soils, Fe(0) or steel dust-activated persulfate oxidation may be a favorable option as an ex-situ remediation process for the treatment of explosive-contaminated soils.

WP246 Stabilization of Heavy Metals in Mine Tailings Using Basic Oxygen Furnace (BOF) Slag

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Mine tailings contain several toxic contaminants, including heavy metals, and the potential is high for deterioration of ecosystems. Inorganic stabilization/solidification processes have been studied to reduce the solubility of heavy metal in mine tailings using binders such as cement, lime, and fly ash. This study considered the possibility of applying stabilization technique to the treatment of mine tailings, using basic oxygen furnace (BOF) slag. The efficiency for stabilization of heavy metals in mine tailings using BOF slag was also evaluated to find the optimum condition. Two mine tailings were used in this study: neutral mine tailing and alkaline mine tailing. The neutral mine tailing (pH 7.0) were highly contaminated with Cu, Zn, Pb, and As, and relatively large amounts of heavy metals leached. Most of heavy metals in the alkaline mine tailing (pH 9.2) were already being stable form, thus did not need to be stabilized. The mineralogical constituents of BOF slag were about 38% of CaO and 29% of Fe_2O_3. Batch slurry test was with 0.5, 3, 5, 10 wt% of BOF slags mixed with 5 g of mine tailings under different moisture contents (i.e., 0.05, 0.1, 0.2, 0.35, 0.5, 0.75, 1 L-water/kg-mine tailing). After agitating for 24 hours under room temperature, and dried in the 65°C oven, then leached by TCLP method to determine the stabilization efficiency. For neutral mine tailings, 10% of BOF slag showed the highest efficiency for cation heavy metals such as Cu, Zn, and Pb (57, 32, 98% of stabilization efficiency, respectively), while 5% of BOF slag did for anion heavy metal including As (81% of stabilization efficiency). Cation heavy metals were highly stabilized with the water holding capacity of mine tailing (0.2 L/kg) under 5 and 10% BOF slag added condition. In the case of As, there was a tendency to be higher stabilization efficiency with decreasing water contents. These differences in optimum condition between cation and anion heavy metals may be due to different stabilization mechanisms. To identify the stabilization mechanism, SEM-EDS and XPS analysis are carried out, and the stabilization efficiency using blast furnace (BF) slag which has a different mineralogical constituents with BOF slag (i.e., about 45% of CaO and 0.8% of Fe_2O_3) was also compared to investigate further mechanism for heavy metal stabilization.

WP247 The re-use of foundry sand by composting – laboratory-scale experiments

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In Europe, around 18 M tons of foundry waste sand is left over from industrial activities every year, and in many cases big landfills do not have the capacity to deal with it. In addition, transport costs to landfills are increasing and alternative ways of treating foundry waste in an environmental friendly way have to be found. To address this, a LIFE Foundrysand project (LIFE13 ENV/FI/2855) piloted a study on how foundry sand waste can be cleaned and hazardous organic substances degraded through novel biological methods. Here we focus on short-term laboratory scale experiments that were run parallel to field scale composting experiments using four different foundry sand types (green, furan, phenolic and their mixture) and composting materials. A 220 L compost system was constructed that consisted of 20-30 % of a foundry sand type and organic materials like wood chips from deciduous trees, horse manure and waste water sludge. The composting process was followed thrice over a period of 8 week by taking samples for chemical and biological analyses, including metals, PAH compounds, fluoride, chloride, sulphates, phenol index, BTEX, DOC, total and soluble N and P, and pathogens (E.coli and Salmonella). Foundry sand specimens showed major differences in their chemical characteristics. Furan sand had the lowest pH and the highest DOC concentration. Phenolic sand had the highest phenol index. Green sand had the highest metal and fluoride concentrations, and a relatively high BTEX level. Our results demonstrated a decrease of the total concentration of PAHs during the experiments. The concentrations of naphthalene, phenanthrene and anthracene were the highest among the 16 PAH compounds analyzed. Fluoride and phenol concentrations were clearly reduced during composting. There were no time-related changes in concentrations of metals, nutrients or BTEX. Salmonella was not detected but E. coli was present i (>1000 cpu/g) in all foundry sand treatments at the end of the experiments. From cleanup and re-use perspectives, our laboratory-scale experiments were only partly successful. Waste water sludge used in the composting was challenging agent because it is characterized by high concentrations of DOC, sulphates and phenols. At the end of the composting tests, these concentrations were above the guideline values, demonstrating an unsuccessful composting process. The final concentration of fluoride and the presence of the pathogens were additional negative indicators.

WP248 Understanding and Evaluating Ecosystem Services at Superfund Cleanups

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Ecosystem services (ES), briefly defined as the contributions of ecosystems to human well-being, are affected by contaminant stressors in the environment and by site remediation activities. Quantifying changes in ES which result from remedial action can inform site management decisions and can show how cleanup supports future ES provisions. This research project utilizes publicly available ES evaluation tools to identify and measure ES endpoints at two pilot Superfund sites representing different ecosystems and scales (a rural watershed-scale setting in the Rocky Mountain west, and a smaller urban setting in the northeastern U.S.). It also highlights how the ES evaluation may be integrated into the ecological risk assessment process. Input for the ES evaluation was derived from land cover maps, reuse planning documents, stakeholder discussions, site visits, and other existing data. The evaluation can help site teams examine relationships between remedial actions and ES, and select best management practices to minimize damage to or improve ES during remedy construction. Evaluation results provide a baseline for any ecological restoration efforts when the pilot sites go into the reuse phase. Outcomes from the pilot sites' evaluations will be codified into a replicable protocol or methodology for ES evaluation at other Superfund sites. Moreover, the ES evaluation protocol will be integrated into Superfund's green remediation strategy. The views expressed in this abstract are those of the authors and do not necessarily reflect the views or policies of the U.S. Environmental Protection Agency.

WP249 Visual Field Characterization of NAPL in Sediment Cores: Developing Standardized Tools Relevant to Assessment and Remediation

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Non-Aqueous Phase Liquids (NAPL) are a frequent target of investigation and remediation for sediment sites contaminated with coal tar, creosote, fuel oils, and other hydrocarbons. In some cases cleanup is required for any sediments containing visually observed NAPL. Such an approach places great importance on the methods used to record observations of NAPL in the field. This approach also differs from that taken for remediation of NAPL in soils, which often differentiates between mobile and residual NAPL. The two-fold purpose of this study is to 1) define the challenges associated with documenting visual presence of NAPL in sediment cores and 2) identify tools that can be used to standardize terminology, field screening methods, and documentation techniques such that they are more relevant to remediation. This study draws from numerous case studies. The study begins by describing typical settings in which NAPL is encountered in sediment and how these settings challenge visual characterization. Based on these settings, simple models of contaminant contribution are developed for several types of NAPL; these models relate NAPL volume and distribution to concentration-based metrics of toxicity and mobility. The study then sets forth tools that can be used to standardize field characterization of NAPL in a way relevant to management of toxicity and mobility. These tools include a framework of terminology that consistently captures NAPL volume and distribution patterns; core collection, processing and photographic methods that minimize impacts of disturbance and maximize visibility of NAPL indicators; screening techniques that help distinguish NAPL from surrounding sediments; and documentation procedures that capture information key to remedial planning. The study identifies recommendations for field characterization methods and potential follow-on work.

Terrestrial or Wildlife Toxicology and Ecology – Poster Only

WP250 Accumulation features of organohalogen compounds and their hydroxylated metabolites in pet cats and dogs: effects on thyroid hormones homeostasis

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The present study determined the levels and accumulation patterns of PCBs, PBDEs, and their metabolites (OH-PCBs, OH-PBDEs, and MeO-PBDEs) in the blood of pet cats and dogs collected from a veterinary hospital in Japan. Furthermore, in order to assess the effects of contaminants in pet cats and dogs, we also examined relationships between the levels of organohalogen compounds and serum thyroid hormones levels analyzed using LC-MS/MS. Median concentrations of PCBs (wet weight) in the cat serum (290 pg g^{-1}) were higher than in dog serum (54 pg g^{-1})

in Japan. OH-PCBs were detected in all serum samples of both dogs and cats (median: 120 pg g^{-1} for dogs and 93 pg g^{-1} for cats). The congener profiles of the OH-PCBs were different for dogs and cats, 3-5Cl OH-PCB congeners (particularly 4′OH-CB18, 4OH-CB25/31/4′OH-CB26) in the cat blood accounted for approximately 90% of the OH-PCBs. In contrast, 5-8Cl OH-PCBs (in particular, 4OH-CB199 and 4OH-CB202) in the dog blood accounted for >90% of the OH-PCBs. Median concentrations of PBDE (wet weight) in the cat serum (630 pg g^{-1}) were higher than in dog serum (150 pg g^{-1}) in Japan. Especially, deca-BDE was the predominant homologue, accouting for 60% of total PBDEs concentrations. Concentrations of OH-/MeO-PBDEs in the serum of dog serum were below the LOQ. On the one hand, OH-/MeO-PBDEs were detected in all the cat serum samples. The major congeners of OH-/MeO-PBDEs identified in both pet food products and blood were natural products (6OH-/MeO-BDE47 and 2′OH-/MeO-BDE68) from marine organisms. In particular, higher concentrations of 6OH-BDE47 than 2′OH-BDE68 and two MeO-PBDE congeners were observed in the cat blood, although MeO-BDEs were dominant in cat foods, suggesting the efficient biotransformation of 6OH-BDE47 from 6MeO-BDE47 in cats. T4, T3 and reverse T3 (rT3) levels showed significant negative correlations with PBDEs and OH-PBDEs concentrations in regression analyses ($p< 0.01$). Especially, DecaBDE was the most affecting isomer for T4, T3, rT3 and free T3 levels, suggested disruption of TH homeostasis. These relationships suggest that the decrease in the levels of T3 and rT3 in the serum of pet cats accompanied with suppression of the T4 in thyroid gland was induced by decaBDE, because T3 and rT3 are produced by the deiodination of T4.

WP251 Assessing Toxicogenomic Effects of 17β-Trenbolone on the Japanese Quail Hypothalamic-Pituitary-Gonadal-Liver Axis

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Trenbolone-acetate is a synthetic anabolic steroid used as a livestock growth promoter. The breakdown product, 17β-trenbolone (17βT), is a known endocrine disruptor; elevated concentrations of 17βT have been reported in solid dung collected from livestock farms. To date, only a limited number of studies have looked at effects of 17βT in birds. Those studies examined sub-lethal effects on the reproductive organs of Japanese quail (JQ) along the hypothalamic-pituitary-gonadal-liver (HPGL) axis and reported reduced numbers of maturing yolk follicles, lowered egg production, decreased plasma testosterone concentrations and changes in mRNA expression of relevant genes such as vitellogenin (VTG). However, these past studies with adult JQ examined only one time point and late in development: 13 weeks post exposure. This missed the initial or earlier exposure period, which might be critical for determining whether the animal compensates for the exogenous endocrine stress, or whether the biochemical/genomic response is inadequate, thereby resulting in adverse effects on reproduction. Furthermore, these previous studies did not look at which changes along the HPGL axis could be specifically linked to changes in egg production. The objective of our research was to build upon previous studies, add 17βT to the adult JQ's diet, and collect samples from the birds on days 3, 7, 14 and 21 allowing us to focus on earlier exposure windows. We analyzed plasma steroid hormone and VTG concentrations, and mRNA expression of HPGL genes such as VTG, 17β-hydroxysteroid dehydrogenase, luteinizing hormone receptor, and CYP19A1, to further characterize the toxicogenomic effects of 17βT on the adult JQ endocrine system. Finally, we determined which changes in these biomarkers can be linked to egg production in JQ and which endpoints are good indicators of egg number and quality. Data collected from this study will also be used to parameterize a computational model that simulates the avian female HPGL axis and vitellogenin production, and relates these biomarkers to the reproductive cycle as well as ecologically relevant endpoints, important for natural resource management and risk assessment.

WP252 Assessment of phytotoxicity (Raphanus sativus and Oryza sativa) of sulfuric acid and nitric acid introduced into agricultural soil by chemical spill

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In Korea, about 44,000 chemicals are used in various chemical industries and transported countrywide. In 2015, 111 chemical accidents occurred and most of the incidents were related to leaks and spills of chemicals from the chemical industry complex or transporters, which can impact nearby soil and terrestrial ecosystem including crops. We assessed phytoxicity of sulfuric aicd (H_2SO_4) and nitric acid (HNO_3), designated as the accident preparedness substances by Korea ministry of environment, contaminated soil using radish (Raphanus sativus) and rice (Oryza sativa), major crops cultivated in Korea. We followed OECD guidelines 208 and 227 for assessing phytoxicity of the chemicals for radish and rice, respectively. Concentrations of the chemicals in aqueous solutions are designated 5 levels including blank. Radish seed were planted in the test soil mixed with the chemical solutions homogeneously and we estimated germination rate and shoot biomass after the test period. We transplanted rice seedlings non-contaminated test soil in pot and the chemical solutions were sprayed into the soil surface. After the test period, we estimated mortality and shoot biomass of the rice. We calculated the toxicity value on the basis of the endpoint observation and measurement during the test period.

WP253 Characteristic for biotransformation of polychrolinated biphenyls in cats

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Hydroxylated polychlorinated biphenyls (OH-PCBs) are formed by the oxidative metabolism of PCBs and PBDEs by cytochrome P450 (CYP) monooxygenase enzyme systems. Our previous study showed congener profiles of OH-PCBs in the blood of cats were totally different from other terrestrial mammals. It is suspected that the thyroid hormone homeostasis is disturbed by these hydroxylated metabolites because of structural similarity with thyroid hormone. However, there are only a few studies on metabolic capacity in cats, and there are many unclear points about the toxic effects and risks of chemical exposures. In this study, we exposed PCBs as model compounds to cats, and analyzed hepatic enzyme activity and gene expressions to collect the foundational information about the metabolic mechanism in cats. Finally, we expect the toxicological effects for thyroid hormone. Difference of residual pattern between high and low chlorinated biphenyls was found. Comparing the results with a similar in vivo experiment conducted in dogs in previously, interspecies differences were found. We also analyzed the change of hepatic enzyme activity and gene expressions of the exposed cats. No change of the activities of the glucuronosyltransferase and sulfotransferase enzymes was detect, although AROD activity (EROD, MROD and PROD activities) was increased, which indicates that the PCBs exposure does not affect the conjugation ability in cats. Also, the expression levels of the CYP1A1 and 1A2 genes were increased, suggesting they were inducted. The expression of the CYP2B genes was unanalyzed because of lack of data base of cats. This study is suggesting PCBs absorption, metabolism and excretion ability of cats differ from dogs, and cats have a higher risk of toxic effects from low-chlorinated OH-PCBs. Especially, it is concern that affects to thyroid hormone homeostasis such as the depressed thyroid hormone levels of cats by PCB exposure, and thus disruptions of thyroid function.

WP254 Characterization of the Effects of Selenium on the Lipid Profiling Induced by Phenyl Mercuric Acetate Exposure in Caenorhabditis elegans

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Mercury (Hg) compounds exposure from environmental and food sources is a significant threat to public health. Studies showed that alkyl compounds of mercury, such as Phenylmercury acetate (PMA) can cause serious damage to the nervous system with sensory and motor deficits syndromes. Recent Caenorhabditis elegans (C. elegans) studies showed that chronic exposure to mercury compounds induces neuron degeneration likely due to the increase in reactive oxygen species (ROS). Selenium (Se) is an essential trace element required for activation of many antioxidant enzymes that have pivotal roles in the brain and endocrine tissues. Accordingly, Se is capable of both decreasing deposition of Hg during co-exposure possibly due to the high affinity between Hg and Se, and reducing the ROS level by activating antioxidant enzymes. In this study, C. elegans utilized as an efficient model system that helps to identify the effect of PMA on the lipid profiling as a proven model for lipid metabolism research. In addition, It can help to reveal the mechanism by which Se associated enzymes may inhibit the toxic effect of PMAby reducing the level of ROS, which reversing oxidative damage in the nervous system. The methodology involved acute and chronic exposure to concentrations of PMA on growth media to investigate the effect of PMA on the lipid profile of C. elegans. Lipid profiling is analyzed by using high-resolution gas chromatography/accurate mass quadrupole time of flight mass spectrometry (GCMS-QTOF). Additionally, the methodology included assessing the oxidative damage of PMA on C. elegans, which involved the probe; 2, 7 – Dichlorodihydrofluorescein diacetate (DCFH-DA). Fluorescence was measured using Spectrofluorometer at excitation 430nm and detection 530nm. Our results suggest that fatty acids profiling, the building block for lipids, may serve as a biomarker for both exposure and effects of environmental toxicant and stress. By utilizing chemical ionization GCMS with accurate mass of the molecular ions we identified the fatty acids profiling of the C. elegans particularly the polyunsaturated fatty acids of the omega 3 type (n-3 PUFAs) such as the C20:5 n-3 and C20:4n-3 which comprising 13.4 % and 2.8% of the total fatty acids in C. elegans respectively. Moreover, results showed a positive relationship between increased concentrations of PMA and the level of ROS in the exposed C. elegans,which eventually affected the fatty acids profiling of C. elegans.

WP255 Comparative in vitro Sensitivity of Amphibian and Mammalian Acetylcholinesterase to Inhibition by Organophosphorus Inhibitors

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Organophosphosphorus compounds (OPs) are used extensively as pesticides worldwide. The acute toxicity for OPs is initiated by inhibition of the enzyme acetylcholinesterase (AChE), leading to accumulation of acetylcholine and disruption of cholinergic signaling throughout the nervous system. A number of studies have reported that amphibians are less sensitive to acute OP toxicity compared to mammals, but the basis for these differences is unclear. Understanding differences in sensitivity to OPs could potentially provide insight into phylogenetic differences in AChE and cholinergic signaling as well as aid in risk assessment. This study compares the in vitro sensitivity of amphibian (Anaxyrus cognatus) and mammalian (Mus musculus) brain and liver AChE to inhibition by paraoxon and chlorpyrifos oxon, the active metabolites of the pesticides parathion and chlorpyrifos. Enzyme sensitivity was evaluated by pre-incubating brain or liver homogenate with one of a range of paraoxon or chlorpyrifos-oxon concentrations (0.001 – 100 µM, 20 min, 37°C), prior to adding substrate (acetythiocholine, 1 mM final) and measuring residual activity. In all cases, AChE in amphibian tissues was markedly less sensitive to inhibition in vitro. The IC_{50s} (concentration that inhibits 50% of enzyme activity) for paraoxon were 275-fold higher (5.3 vs. 0.019 µM) in amphibian brain and 175-fold greater in amphibian liver (8.3 vs. 0.047 µM) compared to that in mammalian tissues. Similar but less extensive differences were noted with chlorpyrifos oxon, i.e., the IC_{50s} for amphibian brain and liver AChE were 31- (136.5 vs. 6.4 nM) and 3-fold higher (128.8 vs. 44.8 nM) than those in the respective mammalian tissues. Substrate kinetics of AChE from the brain and liver in both species were also evaluated. V_{max} was similar between species and tissues, and K_m for liver AChE was also similar between the species. The K_m for mammalian brain AChE, however, was 4-fold lower than that in amphibian brain (0.052 vs. 0.23 µM). These data suggest that phylogenetic differences in AChE and relative sensitivity to OP inhibitors may contribute to observed species differences in acute OP toxicity.

WP256 Compared effect of isolated and combined pesticides on the survival rate in workers of Africanized Apis mellifera (Hymenoptera: Apidae)

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Bees are important pollinating insects and contribute to the maintenance of most natural and agro-ecosystems. However, bees are being affected by different environmental stressors such as habitat fragmentation associated with the increase in intensive agriculture and, consequently, exposure to pesticides on field might be causing the decline of their populations. This study aimed to analyze the effect of thiamethoxam insecticide and picoxystrobin fungicide, isolated and in combination, on the survival rate of Africanized honeybee. Toxicological bioassays by continuous oral exposures were performed with newly-emerged workers (20 individuals in triplicate per group) that were divided in control, solvent-control and the exposed groups: thiamethoxam 1ppb (TXT); picoxystrobin 18ppb (PXT); and TXT+PXT (0,5ppb and 9ppb, respectively), which simulated the dilution of these pesticides in intra-colonial conditions. During oral exposures, two conditions were tested: (I) food with pesticides was offered ad libitum during five days with subsequent substitution by a pesticide-free food until the end of the bioassay; (II) food with pesticides was offered ad libitum during the whole time of bioassay, i.e. twenty-five days. Mortality was recorded daily and survival rates were performed at the end of experiments by means of Kaplan-Meier Survival Analysis: Log-Rank. At the condition I, the results of survival rates showed decreased longevity of bees only for the groups exposed to PXT and TXT+PXT compared to the control group. In the PXT group, bee longevity was reduced to 42.6%, whereas in TXT+PXT group the longevity decreased 23.4%. After twenty-five days of exposure (condition II), the results showed decreased longevity of bees in the groups exposed to TXT, PXT and TXT+PXT compared to the control group. In the TXT group, longevity was reduced to 46.8%, with PXT it decreased 51.7%, whereas TXT+PXT group the longevity decreased 47.8%. Comparing the two tested conditions, oral continuous exposure to sublethal concentrations of pesticides for the period of twenty-five days induced the greatest negative impact on the survival rate of honeybees. Concluding, the results showed the risk of the continuous exposure at sublethal concentrations of these pesticides, indicating that the intra-colonial exposure through food can affect the performance of individuals and, consequently, the homeostasis of the colony.

WP257 Copper toxicity in tropical soil

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Copper is a heavy metal, widely used in the electrical industries and construction, so its presence in the air, water and soil has increased considerably. Copper is considered an essential micronutrient, once it plays an important role in photosynthesis, respiration, perspiration, detoxification, lignification and nitrogen fixation. However, excess of this metal may harm the environment, resulting in reduction of metabolic processes as photosynthesis and growth, reducing the diversity of molds and decreasing the reproductive capacity of animals. The aim of this study was to evaluate the acute and chronic toxicity of copper to the earthworm Eisenia andrei, in two kinds of tropical soils, clayey and sandy, and in an artificial tropical soil (TAS). The TAS was prepared with sand, kaolin and cocoanut fiber, in a proportion of 7:2:1 (w:w:w), respectively. The tropical soils were sampled in areas free of agrochemicals. Copper was incorporated into the soil in the form of a copper nitrate solution prepared with distilled water using eight concentrations, which were 0; 50; 100; 200; 400; 800; 1600 and 3200 mg kg^{-1}, in a volume of water needed to reach 60% of the soil water holding capacity. All the survival and reproduction tests were done with four replicates, using circular plastic containers with approximately 700 g of contaminated soil, 5 g of defaunated horse manure and 10 clitellate worms with an individual body weight of 250-600 mg. Weekly, the humidity was measured and food was provided. The acute toxicity test lasted 28 days with evaluation on the 14th day. The chronic test lasted 56 days with evaluation on the 28th day. Soil parameters as pH, texture, organic matter, copper and cation exchange capacity also were determined in the soils. The sandy soil contained 895 g kg^{-1} of total sand, and presented a pH of 3.9 and 1.5 mg dm^{-3} of copper. The clayey soil presented 295 g kg^{-1} of total sand, pH 4.1 and 1.5 mg dm^{-3} of copper, while the TAS was composed of 810 g kg^{-1} of total sand, with a pH of 5.5 and 2.9 mg dm^{-3} of copper. The LC_{50} and EC_{50} values obtained were 348 and 61 mg kg^{-1} for the sandy soil, 800 and 70 mg kg^{-1} for the clayey soil and 802 and 64 mg kg^{-1} for the TAS. Thus, the acute and chronic toxicity were higher in sandy soil. The lower toxicity observed in clayey soil and TAS probably are due to a higher CTC of the soil, since the smaller particles help to retain copper in the soil, thereby decreasing the bio-available amount.

WP258 Cross omics analysis for PCBs toxicity in the dog brain -Effect on mitochondrial functions-

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Recent studies reported that polychlorinated biphenyls (PCBs) and their hydroxylated metabolites (OH-PCBs) affect the brain nervous system. Dogs are known to have high metabolic capacity toward PCBs and thus may accumulate higher amounts of OH-PCBs in their brain and are potentially at high risk from these compounds. However, there are few studies on the adverse effects of PCBs and OH-PCBs in the brain. The present study performed proteomic and metabolomic analysis of dog (Canis lupus familiaris) brain following PCB exposure. Adult (5-7 months old) male beagle dogs were divided into 3 groups (n=3 each): G1 treated with vehicle control, G2 with PCBs mixture (IUPAC No. 18, 28, 70, 77, 99, 101, 118, 138, 153, 180, 187, and 202). The administration test was performed according to the guideline approved by The Institutional Animal Care and Use Committee. After 5 days of administration of PCBs, all dogs were euthanized and brain samples were collected. In two-dimensional gel electrophoresis, we found 19 significantly up-regulated and 21 down-regulated spots in the G2 and 33 proteins were identified by using MALDI-TOF-MS. In metabolomics, 198 metabolites were detected in the dog brain. Subsequently, partial least squares regression analysis was used to select factors affected by PCBs or OH-PCBs in the brain. 14 (1 positive and 13 negatives) and 21 metabolites (1 positive and 20 negatives) were correlated with the concentrations of PCBs and OH-PCBs, respectively. These metabolites were those involved in mitochondria functions such as urea cycle and glycolytic system/gluconeogenesis. In addition, ATP and ADP were significantly down-regulated by PCB exposure. Proteomic analysis showed a decrease of NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 8 (NDUFA8); which is one of the protein constituents of complex I in the inner mitochondrial membrane. Complex I transfers electrons from NADH to the respiratory chain and simultaneously creates a proton gradient across the inner membrane that powers complex V to generate ATP. Therefore, proton gradient might be disrupted. PCB exposure also down-regulated the expression of proteins related to mitochondria functions including ACSBG1, HADHA and SLC25A12. These results suggest that PCBs and/or OH-PCBs might inhibit mitochondria functions in the brain.

WP259 Declining surf scoter populations in Puget Sound: Capturing the signal from multiple sublethal endpoints

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North American populations of surf scoters (Melanitta perspicillatta) and white-winged scoters (Melanitta fusca) have declined approximately 60% over the last 40 years. Food resources, selection, and availability explain some but not all of the population decline. It is intuitively clear that coping with contaminants redirects resources from growth and reproduction, affecting fitness and ultimately, population dynamics. Thus, we hypothesized that accumulation of cadmium, mercury, and selenium in kidney and liver predict body condition. Adult males were collected in December 2005 and March 2006 from three Puget Sound locations. Response variables included four aspects of body composition (mass, lipid, protein, ash) and three plasma metabolites (triglycerides, butyrate, and uric acid). Both predictor and response variables had non-normal distributions with unequal variances. Using the software PRIMER-E, we present the novel approach of several using multivariate, permutational methods. More importantly, we were able to integrate the seven response variables into "signature" centroids that were unique for each individual. Overall, scoters in this study contained low contaminant levels below those that cause pronounced damage in mallards. All sea ducks trended to lower mass and generally poorer body condition in spring. Distance-based Linear Models (DistLM) showed that among Surf Scoters, Se and Cd in liver and kidney explained 21% of the variance in their body condition. DistLM further showed that despite seemingly high correlations with Cd and Se in an ordination analysis, these contaminants did not significantly explain body condition in White-Winged Scoters. Surf Scoters are approximately 60% smaller than White-Winged Scoters. Smaller species have increased basal metabolism, higher costs of thermoregulation and locomotion, and store less energy. Distinct interspecific response to contaminants is consistent with greater diversion of resources from body condition. Subtle sublethal responses among surf scoters indicate the need for innovative statistics that capture what is ultimately a substantial amount of variation in their body condition, which is analogous to reproductive fitness. Such improvements in signal recognition provide critical insights into the contribution of sublethal stressors to population declines.

WP260 Dermal Uptake of Organic Contaminants by Amphibians Based on Location of Exposure and Hydrophobicity of Contaminant

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Dermal exposure, which is likely the dominant uptake route for amphibians for most contaminants, is likely dependent on location of exposure and hydrophobicity of the contaminant. The objective of this study was to characterize both ventral and dorsal dermal uptake of contaminants across a range of hydrophobicities in Blanchard's cricket frogs. Amphibians were dosed with contaminants (atrazine, lindane, fluoranthene, benzo(b)fluoranthene, and bifenthrin) suspended within the Sniper® insecticide formulation (AI: bifenthrin) applied using a dermal patch system on either the ventral or dorsal side. Individuals were collected at specific time points, sacrificed, and prepared for analysis. Excised exposed skin samples and remaining carcass were processed separately using the QuEChERs method and analyzed using GC/MS. Comparable data between ventral and dorsal contaminant movement allows risk assessors to note differences in availability depending on the site of exposure and how ventral and dorsal skin locations differ in their taking up of hydrophobic contaminants from a hydrophobic vehicle.

WP261 Development of a Behavioral Assay to Test Pesticide Toxicity in Amphibians

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Prey capture in toads consists of a stereotypical series of behaviors beginning with orientation toward the prey item, followed by pursuit and striking. Prey-orientation behaviors have been used to explore neurological function in toads; however, orientations toward the stimulus decrease over time, indicating toads habituate to the stimulus when behaviors are not reinforced. The goals of this study were to characterize prey-orientation behaviors and develop an assay for use as an indicator of toxicity from pesticide exposure, and then verify the effectiveness of the behavioral model by examining the effects of chemicals on prey-orientations. Because prey capture is dependent on neurological function, exposure to compounds that inhibit or stimulate neurotransmitters may influence the prey capture sequence, including prey orientation, strike accuracy, and endurance. Thus, we first examined whether reinforcement of prey-orientation behaviors influences habituation in sub-adult Great Plains toads (Anaxyrus cognatus). Results indicate that habituation occurred when the toads were not 'rewarded' after testing. Therefore, without motivation to capture a prey stimulus, prey-orientations decreased over time. Furthermore, we were also able to use different time intervals and 'rewards' to establish a system where orientations were consistent and maintained long term. Our results will be discussed relative to the usefulness of prey-orientation behaviors as indicators of toxicity following environmentally realistic, worst-case exposures to chemicals.

WP262 Diethylhexyl phthalate increases deposition of 14C-bisphenol A in reproductive tissues of mice (Mus musculus)

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Xenoestrogens, estrogen mimicking chemicals, are widely used in many personal care products and containers. This widespread use exposes a large percentage of the population in developed countries to these chemicals through inhalation, ingestion, and skin absorption. Two popular xenoestrogens, bisphenol A (BPA) and diethylhexyl phthalate (DEHP), have been shown to disrupt blastocyst implantation. Recent research has indicated that concurrent administration of BPA and DEHP can reproduce these effects at a lower dosage. This may caused by DEHP increasing available BPA in reproductive tissues, thus requiring less of the chemical to exert an effect. Consequently, we sought to test BPA deposition in various peripheral tissues by injecting 0, 3, 9, or 18 mg DEHP into female and male CF-1 mice followed by 50 µg/kg ^{14}C-BPA through oral consumption. Animals were dissected 1 h following ^{14}C-BPA administration and various tissue samples were acquired. Samples were solubilized and results were obtained through a liquid scintillation counter. Results indicated that the 18 mg and 9 mg DEHP doses magnified BPA deposition in the uterus and ovaries relative to controls, in both cycling and peri-implantion females. The 18 mg dose also increased BPA deposition in the epididymides in males, with similar trends increasing with DEHP dose in other reproductive tissues. These results demonstrate that DEHP can synergize with BPA in estrogen-binding reproductive tissues.

WP263 Effect of Exposure to Pesticide Mixtures on Body Burden and Metabolomic Profiles in Amphibians

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The agricultural use of pesticides to control undesirable species has gained momentum in recent years, despite a growing body of research highlighting the potential adverse ecological effects of these chemicals. Multiple pesticides are often applied to fields in combination through tank mixtures, in an effort to minimize multiple pests' impacts, cost and time spent on control measures. Many non-target organisms, such as amphibians, also come into direct contact during application or indirect contact with pesticide contaminated soil and plants in agricultural areas. While individual pesticides are known to have adverse effects on amphibians, research on multiple pesticide co-exposures in terrestrial phase amphibians is lacking. The purpose of this study was to compare amphibian body burdens and hepatic metabolomic profiles after exposure to a single pesticide or pesticide mixtures to better understand the possible synergistic, antagonistic or additive effects these exposures induce. In total, five common-use pesticide active ingredients were used in this study, three herbicides (atrazine, metolachlor and 2,4-D), one insecticide (malathion) and one fungicide (propiconazole). Juvenile southern leopard frogs (Lithobates sphenocephala) were reared from egg masses to 30-60 days post-metamorphosis. Individual frogs were then exposed to a single pesticide, a combination of herbicides (atrazine, metolachlor and/or 2,4-D) or a combination of different classes of pesticides (atrazine, malathion and/or propiconazole) at the labeled application rate on low organic matter soil. Following 8 hr exposures, liver samples were excised for metabolomic profiling and body burdens determined from whole tissue homogenates. Body burden results indicate that amphibian accumulation of multiple pesticides occurs synergistically between certain compounds. Metabolomic profiling of livers support this and pesticide mixtures appear to also synergistically influence biochemical perturbations in these non-target species. Co-exposure to multiple pesticides in amphibians may enhance uptake and decrease normal metabolic function, thereby limiting the overall health and stability of amphibian populations in agricultural areas.

WP264 Effects of a glyphosate end-use product on the flowering of black-eyed Susan (Rudbeckia hirta) and red clover (Trifolium pratense)

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It has long been recognized that standard terrestrial plant toxicity tests are on their own insufficient for assessing the risk of pesticide use to non-target terrestrial plants. Current standard tests only consider a small fraction of a plant's life cycle (seedling emergence and vegetative vigor), and they only involve early life stage exposures. Understanding the overall effects of realistic plant exposures to pesticides is critical for an accurate ecological risk assessment. Initial test results with black-eyed Susan (Rudbeckia hirta) and red clover (Trifolium pratense) have indicated early bud-stage plant exposure to glyphosate may cause flower deformation at relatively low exposure levels (1% of application rate). We will present our findings of current follow-up experiments with the same species over a wider range of exposure levels, in an effort to resolve rate-response relationships for effects on flower phenology and morphology in these species.

WP265 Embryonic Origins of Altered Ovarian Gonadotropin Responsiveness in an Environmental Model of Endocrine Disruption, the American Alligator

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Exposures to endocrine disrupting contaminants are thought to broadly impact reproductive health in humans and wildlife. As an environmental model of endocrine disruption, the American alligator has provided significant utility to our understanding of population-level effects of exposure to endocrine-active chemicals on vertebrate reproduction.

Reports from a contaminated lake (Lake Apopka, FL) have indicated a causal role for these contaminants in the rapid decline of alligators living there in the 1980s-90s. Juvenile alligators from this site are characterized by genital abnormalities, aberrant sex-steroid hormone production, and abated ovarian responsiveness to gonadotropin signals. Consistent with a "developmental origins of adult disease" model, studies from our lab have provided evidence that this collective reproductive disorder has embryonic origins. We seek to further understand the mechanism(s) underlying these observations by testing the hypothesis that precocious exposure to hormone signals during critical windows of embryonic development is responsible for suppressed gonadotropin response later in life. We have exposed alligator embryos to 17β-estradiol or dihydrotestosterone at a stage just prior to sex-determination and raised resulting hatchlings for five months prior to administering exogenous ovine follicle-stimulating hormone (FSH). We aim to investigate gonadotropin responsiveness by: quantifying induction of transcriptional targets critical to ovarian function, increases in gonadosomatic index (GSI), and by examining cellular dynamics of FSH action at the histological level. Utilizing multiple regression modeling, we have established induction of ovarian increases to GSI and gene expression of eleven targets in response to FSH-stimulation in control and treated animals from a contaminated site, Lake Apopka, and a reference site, Lake Woodruff. We have further detected significant impacts of site and developmental exposure on expression of four nuclear receptors, estrogen receptors α and β (ESR1, ESR2), and arylhydrocarbon receptor isoforms AHR1A and AHR2. Incidental or experimental exposure during development to endocrine-active compounds significantly modulates the response of these genes to FSH in a manner not observed in control animals. Given the broadly conserved role of these genes in vertebrate reproduction, these findings implicate developmental endocrine disruption in altered gonadotropin responsiveness later in life.

WP266 Evaluating the terrestrial toxicity of hydrophobic contaminants using a standardized soil mixing and aging procedure

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Environmental risk assessments require quality toxicity data to provide defensible environmental quality benchmarks. Hydrophobic organic compounds (HOCs) are often difficult to test due to multiple compound loss processes (sorption, volatilization, biodegradation, etc.) and slow kinetics. Poorly performed exposures with little to no equilibration between the test material and soil media often confound test result interpretation. Proper soil mixing and aging prior to the in-life phase of a terrestrial toxicity experiment is critical to minimizing artifacts of physical effects of neat test material that had not fully equilibrated with the test media. The study objectives were to 1. Develop an aging procedure which could be used to accurately and consistently characterize the hazard of HOCs in soil, 2. Apply the aging procedure to chronic toxicity tests with terrestrial plants (Chronic plant toxicity test with Brassica rapa and Avena sativa) and soil invertebrates (28-d survival and reproduction test with Folsomia candida) and assess effects, 3. Validate the Target Lipid and Equilibrium Partitioning Model TLM-EQP for deriving Predicted No Effect Concentrations (PNECs) in soil for HOCs. Compounds tested ranged in log K_{OW} values of 5-9. Terrestrial plant endpoints observed consisted of shoot weight (wet and dry), number and weight of inflorescences. Soil invertebrate endpoints observed consisted of adult mortality and number of young produced. The datasets generated here demonstrate that the TLM-EQP models are protective of these endpoints and support efficient use of data for general purpose risk assessments.

WP267 Factors Affecting Arbuscular Mycorrhizal Fungal Colonization in Soils Contaminated with Heavy Metals

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High concentrations of heavy metals in soils have been shown to adversely affect microbial and plant populations. Arbuscular mycorrhizal fungi (AMF) colonize the root cortex of certain plant species, engaging in nutrient exchange and facilitating a symbiotic relationship. This relationship becomes particularly important when considering plant growth on severely disturbed and contaminated sites. This study examined how the AMF status of pre-existing plant communities affects the microbial colonization of newly arriving plant species and how aboveground plant competition affects those AMF dynamics in soils contaminated with heavy metals. C4 grasses and sugar maple trees rely heavily on AMF, while grey birch trees are thought to more commonly form relationships with another type of mycorrhizal fungus, ectomycorrhizae. AMF colonization was tested against two independent factors: the type of vegetation characterizing the area (grey birch or C4 grass) and the status of that vegetation (intact or cleared). Clearing the plots helped to isolate the soil effect of interest from potential differences in aboveground competition between grasses and birches. Sugar maples, representing later-successional trees that one might expect to follow the birches naturally in succession, were planted in these experimental soil conditions. In this study of 44 root samples, AMF colonization was assessed by looking at several separate microbial structures. The percent colonization by AMF showed a statistically significant increase in areas of C4 grasses relative to grey birch soils. For those microbial structures where there was significant interaction between vegetation type and status, areas cleared of grasses showed the highest AMF colonization. This suggests that C4 grasses create a pre-existing mycorrhizal community in which the symbiotic relationship between AMF and sugar maples can flourish, even in heavy metal environments, in a way that grey birch does not. However, competition with intact grasses may reduce AMF colonization rates. These findings become important when considering phytoremediation practices and identifying the most effective plant and microbial treatments for a given environmental problem.

WP268 Factors Affecting the Toxicity of Headline® Fungicide to Terrestrial Toads

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Previous studies have demonstrated that pyraclostrobin-based fungicides (e.g., Headline® and Headline AMP® fungicides, BASF) are toxic to some amphibians at environmentally relevant concentrations. However, various factors influence exposure and effects in an environmentally realistic exposure scenario. These include age of the individual, exposure route, and physiological state of the individual. Further, there are no data available on the sublethal effects of pyraclostrobin-based fungicides in terrestrial amphibians. Thus, we investigated factors affecting toxicity of Headline formulations to amphibians, including body size, exposure route, and hydration state of the animal. Additionally, we examined sublethal effects of Headline AMP fungicide using prey-orientation behaviors as indicators of toxicity. Neither Headline nor Headline AMP fungicide caused mortality of adult Great Plains toads (Anaxyrus cognatus)and Woodhouse's toads (A. woodhousii) with overspray exposures up to 5x the maximum label rate for North American corn. Exposure to Headline AMP via previously treated soils caused dose-dependent mortality of juvenile A. cognatus when toads were placed immediately onto treated soils, but no toxicity was observed when toads were placed onto treated soils 60 and 120 min post-treatment. Hydration state did not influence the toxicity of Headline AMP to juvenile A. cognatus (LC_{50}= 2.4 and 2.3 µg pyraclostrobin/cm^2 in dehydrated and hydrated treatments, respectively). Finally, orientations toward a simulated prey item by juvenile A. cognatus decreased over time; however, Headline AMP did not influence the number of orientations toward a simulated prey or the rate at which orientations toward the stimulus changed over time. Therefore, risk from Headline fungicides to terrestrial amphibians in a routine crop setting appears generally low.

WP269 Factors Influencing Mallard Duck (Anas platyrhynchos) Fertility in the Avian Reproduction Test

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In 2012, the Avian Reproduction Test guideline (OCSPP 850.2300) was revised stating that validity criteria for mallard duck fertility (i.e. number viable embryos of eggs set) in the control group must achieve 80%. Historical data suggests that achieving 80% fertility may present significant challenges as a result of the incompatibility in mating pairs. Courtship and pair formation are of great importance to waterfowl populations with many duck species forming pair bonds each breeding season (seasonal monogamy). These courtship factors suggest that female ducks will tend to select their mates prior to the breeding season which heavily influences successful copulation and egg fertility. In addition, research has shown that females have developed elaborate behavioral and anatomical sexual strategies to resist forced copulation of unwanted males. The research presented here reinforces these concepts of mallard hens either accepting or rejecting the males, or males being deficient in producing fertile eggs during the avian reproduction test. Laboratory trials were conducted with mallards in the reproductive phase in which unsuccessful males (those not producing any fertile eggs) were replaced with proven breeder males in cages with female hens. Conversely, the unsuccessful males were paired with fertile females to determine to what extent fertility would be negatively impacted. To eliminate the variable of infertility attributed to regressed testes or ovaries, necropsy was performed on all birds at test termination. Additional factors influencing mallard duck reproduction including age of birds, compatibility of mating pairs, and randomization of pairs will be discussed.

WP270 Female vinegar flies reared in leaded environments do not avoid leaded (Pb2+) egg-laying substrates

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Research has shown that female vinegar flies (Drosophila melanogaster) prefer to lay eggs on sugar-based agar medium and that preference is linked to efforts to decrease larval foraging costs.Therefore, females would likely selectively prefer egg-laying substrates devoid of potential harmful toxins, such as heavy metals, which could potentially decrease offspring fitness. The purpose of this research was to determine if females would avoid or prefer leaded medium as egg-laying substrates, based upon their development in control or leaded medium. Wild type D. melanogaster were reared on control or leaded (250, 500 or 1000 µM PbAc) medium from egg stage to adulthood. Females were mated, provided three substrates to lay eggs (control, acetic acid, and leaded medium) and the number of eggs laid on each substrate was counted. There was a dose-dependent response in the mean total number of eggs laid by females ($p = 0.05$), but not for female preference for control, acetic acid or leaded egg-laying substrates. However, control females preferred to lay eggs on control substrates ($p < 0.05$) and avoided leaded substrates ($p < 0.001$) whereas leaded females (in any dose) did not exhibit a preference for either substrate ($p > 0.05$). These results indicate that females reared in a leaded environment may be unable to determine the toxicity of egg-laying substrates. The ecological consequences of laying eggs in a lead-polluted environment include potential increased larval foraging costs for offspring, as well as bioaccumulation in the food chain and multi-generational accumulation within the population.

WP271 Heavy metal lead induced aberrant expression of microRNAs in cotton

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MicroRNAs (miRNAs) play key roles in plant responses to various metal stresses. To investigate the miRNA-mediated plant response to heavy metals, cotton (Gossypium hirsutum L.), the most important fiber crop in the world, was exposed to different concentrations (0, 25, 50, 100, and 200 mg/L) of lead (Pb) and then the toxicological effects were investigated. The expression patterns of sixteen stress-responsive miRNAs and 10 target genes were monitored in cotton leaves and roots by qRT-PCR; of these selected genes, several miRNAs and their target genes are involved in root development. The results show a reciprocal regulation of cotton response to lead stress by miRNAs. The characterization of the miRNAs and the associated target genes in response to lead exposure would help defining the potential roles of miRNAs in plant adaptation to heavy metal stress and further understanding of miRNA regulation in response to abiotic stress.

WP272 Hepatotoxicity of gasoline fume inhalation in albino rats

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Hepatotoxic and Genotoxic effects associated with gasoline fumes were assessed in forty Albino rats following inhalation exposure. The rats were randomly assigned to five experimental treatments (T) with eight rats per treatment (T1, T2, T3, T4 and T5). The control treatment, T1 was housed in a section of experimental animal house free from gasoline fumes while T2, T3, T4 and T5 were exposed to gasoline fumes in exposure chambers for one, three, five and nine hours daily respectively for a period of twelve weeks. Serum alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Alkaline phosphatase (ALP) and histopathological examination of the liver tissues of each treatment were used as diagnostic markers to assess liver dysfunction. Genotoxicity test on the lung tissues of each treatment was conducted based Randomly amplified polymorphic DNA fingerprinting polymerase chain reaction (RAPD PCR) technique. Significant increase ($p< 0.05$) in the level of ALT, AST and ALP for T2, T3, T4 and T5 as compared to T1 were recorded. Photomicrograph examination of the liver sections of T1 showed hepatic tissue with normal liver cell architecture while that of T2, T3, T4 and T5 revealed degenerative changes in the ultrastructural integrity of the hepatic cells. Genotoxicity test revealed DNA bands at a reducing intensity from T1 to T5. The suspected DNA damage as compared to T1 followed the trend: T3>T5>T4>T2. Similarity between the DNA bands of the experimental rats was followed by rats exposed to T2, T4 and T5 and T3 respectively. In conclusion, frequent exposure to gasoline fumes may induce hepatoxicity and genotoxicity, hence impairing the normal liver function and gene structure.

WP273 High throughput screening for pesticide-induced mitochondrial dysfunction

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Pesticides include the broad classes of herbicides, insecticides, and fungicides. While pesticides can elicit adverse effects in non-target organisms through different mechanisms (i.e. endocrine disruption, neurotoxicity, tissue necrosis), mitochondria can also be a prominent target for toxicity. Mitochondria serve multiple roles in the cell, including ATP production, calcium regulation, and apoptosis. Pesticides can act to impair mitochondrial bioenergetics, which results in energy deficits and disruptions in downstream cellular processes. In this study, we tested pesticides from different classes to establish whether these chemicals act in a common way to disrupt mitochondrial bioenergetics. The overarching goal is develop a high throughput screening assay for pesticide-induced mitochondrial dysfunction and to determine the underlying mechanisms of mitochondrial toxicity. We tested (1) Fipronil, a broad-use insecticide that belongs to the phenylpyrazole family, (2) Fluazinam, a broad-spectrum fungicide classified as an arylaminopyridine, (3) Itraconoazole, a triazole antifungal used as a medication to treat fungal infections, and (4) Paraquat, a redox-active heterocycle herbicide that is structurally similar to compounds that induce Parkinson's disease. Rat N27 dopaminergic cells were treated for 24 hours with a dose range of 0.25, 2.5, 25, or 250 µM. Using the Seahorse XFe24 extracellular flux analyzer, cells treated with 25 µM of all 4 pesticides inhibited basal respiration of mitochondria. N27 cells treated with 25 µM pesticide also showed lower maximum respiration following treatment with the uncoupling agent carbonyl cyanide p-trifluoromethoxyphenylhydrazone (FCCP), the most dramatic response being that of fipronil (~25% decrease compared to ~50% for the other compounds). Noteworthy was that PQ2+ reduced "spare respiratory capacity" of the mitochondria, which is the difference between ATP produced by oxidative phosphorylation at basal and maximal activity. Pesticides resulted in different cell energy phenotype profiles, suggesting different mechanisms of action on mitochondria. Each of the four pesticides were demonstrated to impact mitochondrial bioenergetics, but some pesticides were stronger mitochondrial toxicants (i.e. fipronil) than others. Future objectives include identifying the mechanisms of mitochondrial dysfunction in cell lines and whole animals, to determine the relationship between pesticide exposures and increased risks of neurodegenerative diseases.

WP274 Lead-induced physiological and biochemical changes in cotton

Q. He, East Carolina Univ; B. Zhang, East Carolina Univ / Biology

Heavy metal lead (Pd) is one major environmental contaminant, which exists anywhere around us and is causing significant environmental and human health problem. Although there are much study on animal health, few studies has been performed on the impact of plants. In this study, we systemically investigated the effect of lead in the aspect of plant physiological and biochemical change. Lead exposure significantly inhibited cotton plant growth and development in the aspect of both fresh and dry biomass yield of both roots and aboveground parts with a dosage-dependent manner ($p< 0.001$). Lead treatment inhibited root development potentially by damaging root apical meristem and root cell elongation. Low concentration lead promote seedling water contain; but this only affected aboveground parts not the roots. Lead exposure significantly inhibited the biosynthesis of chlorophyll a, chlorophyll b and total chlorophylls ($p< 0.001$) and further affected the photosynthesis of cotton seedlings. Lead exposure caused oxidative damage in cotton seedlings evidenced by cell membrane damage and the expression profile changes in oxidative stress-related enzymes. The contents of the indicator of membrane damage, malondialdehyde (MDA), were significantly increased under Pb stress exposure. All tested anti-oxidative enzymes, SOD, POD and CAT, was induced at low lead concentrations and then inhibited at high concentrations with a peak at 50 µM. This suggests that cotton has an oxidative stress protection system to prevent heavy metal-caused oxidative damage.

WP275 Mercury contaminated food decreases the growth of collembolans: A bioaccumulation and growth study

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In the terrestrial environment, mercury (Hg) is subject to redistribution and transformation into different inorganic and metal – organic species that are uptake by vegetation and soil organisms. In the present study, we assessed the effects of food contaminated with relevant concentrations of Hg in the form of $HgCl_2$. Food was provided ad libitum to a soil dwelling representative invertebrate, Folsomia candida. Changes in growth rate and Hg bioaccumulation levels were assessed at different concentrations of Hg in food providing data that could complement the effects of Hg on reproduction and survival using standardized organisms and protocols. In this study, collembolan growth was dependent of the Hg food concentration. At all the studied concentrations the growth of collembolans was affected by the Hg in yeast with differences in growth rate of organisms. Also, the length of animals were statistically different for all treatments at the end of the test, comparing with the control. Collembolan growth was recorded every two days, and a Von Bertalanffy's growth curve was derived along with growth rate. The toxicokinetics patterns at the exposure concentrations of Hg in food were not significantly different from each other. It is advised that growth tests could be seen as an important step to fill the gaps not only of the bioaccumulation tests, but as well of the advised chronic reproductive assays that could explain some effects. Even at low and environmental relevant concentrations of Hg, collembolans suffered differences in their growth rate, which could pose a risk to ecosystems, knowing the important role of these kind of organisms. Also, this study highlights the importance of these complementary tests for a better and complete approach in risk assessment studies.

WP276 Metal Accumulation in Terrestrial Animals

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Metals are commonly used in many facets of modern society and are thus frequently released into the environment. Metal accumulation in wildlife may occur as a result of excess metals in the environment, which in turn can lead to toxicity when concentrations exceed certain threshold levels. Unfortunately, tissue metal concentrations in many terrestrial mammals are to a large extent unknown. Here, we present the first analyses of liver metal concentrations in 6 species of mammals collected over four years from four sites in Georgia and Florida that experienced relatively similar levels of human disturbance. Liver tissues were obtained from hundreds of armadillos, bobcats, coyotes, opossums and raccoons, and were screened for multiple metals. Differences in metal concentrations due to age (juvenile versus adult), sex, site and time (yearly variation) were examined. This study provides the first extensive reference data for metal concentrations in various key wildlife species found in terrestrial habitats. Results of this study will also increase understanding of metal accumulation and transfer in terrestrial food webs.

WP277 Mitochondrial bioenergetics are impaired by the legacy pesticide dieldrin

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Mitochondria are direct targets of chemicals in our environment. Toxicants can modify mitochondrial function via a number of mechanisms including DNA damage, electron transport chain inhibition/uncoupling, and generation of reactive oxygen species, among others. Dieldrin (DLD) is a legacy organochlorine pesticide (OCP) that bioaccumulates in tissues and is epidemiologically associated to an increased risk for Parkinson's disease (PD). This study aimed to determine whether DLD impacts mitochondrial bioenergetics in dopaminergic neuronal cells, testing the hypothesis that DLD decreases oxygen consumption rate and overall performance of the mitochondria. Due to the association with Parkinson's disease, immortalized dopaminergic N27 cells were treated for 24 hours with one dose of either a solvent control or one dose of 2.5, 25, or 250 µM DLD. Cells treated with 250 µM showed higher apoptosis (caspase 3 activity) relative to control cells. Cell viability decreased with 25 and 250 µM DLD, but there was no cytotoxicity with DLD at the doses and time point examined. Following treatments, viable cells were subjected to a mitochondrial stress test using the Seahorse XFe24 extracellular flux analyzer. N27 cells treated with 25 µM DLD showed lower maximum respiration compared to the solvent control following treatment with the uncoupling agent carbonyl cyanide p-trifluoromethoxyphenylhydrazone. There was also evidence of lower spare respiratory capacity in mitochondria with treatments >25 µM. Studies are ongoing to measure the expression profiles for transcripts related to oxidative phosphorylation (e.g. cytochrome C), oxidative stress (e.g. superoxide dismutase), and mitochondrial membrane polarization and permeability (e.g. Bcl2, TSPO, and voltage dependent anion channel), to determine the mechanism associated with impaired bioenergetics. In order to increase the relevance of the study to PD, tyrosine hydroxylase (TH) protein abundance was measured. TH did not differ among groups, suggesting that impaired mitochondrial respiration may not directly impact DA synthesis. This study suggests that impaired mitochondrial bioenergetics may, in part, underlie the relationship between pesticide exposures and neurodegenerative diseases.

WP278 Movement of Hydrophobic Organic Contaminants from Soil into Amphibians Based Upon Soil Characteristics

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Limitations in our understanding of dermal uptake of pesticides adds to the difficulty of incorporating amphibians into the ecological risk assessment process. This study seeks to characterize the degree to which soil-contaminant aging and soil organic carbon content affect the uptake of five moderate to highly hydrophobic contaminants into amphibians. Amphibians were exposed to Sniper® formulation (AI: bifenthrin) spiked with atrazine, lindane, fluoranthene, and benzo(B)fluoranthene. Low and high organic carbon soil was sprayed with this spiked formulation and amphibians were introduced at either 10 minutes after spray or 4 hours. Individuals were collected at specific time points, sacrificed, and prepared for analysis. Whole body amphibians samples were homogenized and processed via the QuEChERs method and analyzed using GC/MS. Information on the uptake of soil contaminants into an amphibian will not only be valuable in assessing the magnitude of uptake in general, but will also allow us to visualize the time course of contaminant uptake and allow us to characterize the effects of soil OC on soil-contaminant aging and amphibian uptake.

WP279 Polycyclic Aromatic Hydrocarbons metabolites in bile and serum from resident sea birds (F. magnificiens, S. leucogaster, L. dominicanus) from Brazil

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Resident seabirds can be used for biodiversity monitoring programs because these animals are vulnerable to pollution, such as Polycyclic Aromatic Hydrocarbons (PAHs) from oil and derivatives. Seabirds assimilate PAHs by the dermis contact with contaminated water, and sediment, and contaminated particles ingestion, , ending up in the blood stream and being mostly metabolized in the liver and secreted by the gall bladder through a hepatobiliary duct. There is evidence that vertebrates have a system of xenobiotics metabolizing enzymes highly developed, located by the cytochrome P-450, able to metabolize PAHs, secreting or absorbing them from the intestinal tract. The aim of the present study was to compare the PAHs metabolites in serum and bile from resident sea birds (Fregata magnificiens, Sula leucogaster, Larus dominicanus) from the state of Rio de Janeiro, Brazil, in specific oil exploration and urban occupation areas, as the Campos Basin region. The samples of serum (n=19) and bile (n=24) were collect from animals that were either found dead during beach monitoring procedures or died at rehabilitation center, located in Araruama city, between 2015 and 2016. The metabolites were analyzed with a high performance liquid chromatograph with fluorescence detector (HPLC/F) and quantified as Naphthalene (NAP) Phenanthrene (PHE) and Benzo(a)pyrene (BaP) equivalents. In only three individuals (two S. leucogaster and F. magnificiens) it was possible to collect serum as soon as the animal arrive and bile after their death. The results of all samples demonstrated that the concentration of the serum were almost one order of magnitude lower than the bile. The concentration of the metabolites was NAP >PHE>BaP for both serum and bile. The majority of the bile samples have presented higher levels than those found in preliminaries studies with migrate seabirds, such as Magellanic penguins stranded in the Brazilian coast.

WP280 Relationship between body condition and contaminants in Overwintering Canvasbacks (Aythya valisineria) in the Lake St. Clair/St. Clair River Area

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Overwintering canvasbacks were collected in the Lake St. Clair/St. Clair River area in the winter of 2008/09 and 2010, and livers were analyzed for PCBs, organochlorine pesticides, brominated flame retardants, mercury (Hg) and selenium (Se). Hepatic concentrations of organochlorines, brominated flame retardants, and Hg in all birds were low and Hg concentrations were below levels associated with adverse effects on survival.Hepatic concentrations of Se were largely low but were more variable among birds with 25% of canvasbacks showing concentrations considered elevated and 5% (i.e., one bird) with a concentration exceeding the threshold associated with toxicity. Significant increases in hepatic concentrations of Hg and Se, sum PCBs, p,p'-DDE and other organochlorines were evident over the four month period of collection in which mean concentrations in January and/or February were significantly higher than mean concentrations in November when overwintering birds arrived in the study area. The largest increases were most often between December and January which also coincided with the period when birds from Lake St. Clair moved, following freeze-up of the lake, to the St. Clair River to forage. Simultaneous with the increase in body burdens, body condition of the birds declined from December onward. Although it is unclear to what extent possible changes in diet, level of contamination and/or declines in lipid reserves following movement to the St. Clair River contributed to observed temporal trends in body burdens of overwintering canvasbacks, our evidence suggest that a reduction of body condition, through loss of fat reserves, may have contributed to the higher body burdens in winter in canvasbacks.

WP281 Risk assessment of lead exposure from recreational fishing tackle to nestling bald eagles in a heavily utilized recreational fishery

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Risks associated with lead exposure in wildlife are well documented and have been found to cause physiological dysfunction, development deformities, and mortality. Opportunistic feeding avian species, such as bald eagles (Haliaeetus leucocephalus), have most often been associated with lead exposure from spent ammunition left behind in the offal of big game and formerly lead shot from wounded or lost waterfowl. The U. S. Fish and Wildlife Service banded lead shot for waterfowl hunting in the 1980's and many state agencies are beginning to phase out the use of lead for big game hunting for non-toxic alternatives. However, other sources of lead exposure such as that from lost or improperly discarded recreational fishing tackle still exist. We initiated a field study and retrospective analysis to assess the risk of recreational fishing tackle to nestling bald eagles in a heavily utilized recreational fisheries. Voyageurs National Park (VNP) in Northern Minnesota receives up to 750,000 angler hours per year. Our study had two objectives, 1) determine the blood lead concentration of nestling bald eagles in VNP, and 2) assess the exposure risk of nestling bald eagles to recreational fishing tackle, especially fishing tackle that could result in lead exposure. In 2013, we samples 25 nestling bald eagles for blood lead concentration and found 24 were below the 3.3 ug/dl detection limit. The only nestling greater than the detection limit had a blood lead concertation of 6.0 ug/dl. We also scanned all nestlings sampled with a BlueMax 950 Metal Detector to determine if nestlings had recently ingested lead and found no detectable lead fragments within nestlings. To assess the exposure risk of nestling to recreational fishing tackle we conducted a retrospective analysis of banding records from 1989 to 2013 for the documentation of fishing tackle and any harmful effects of tackle. We found no observable incidences of acute lead toxicosis that might be associated with ingestion of lead fishing tackle. However we did find that 10.3% of all nests visited had fishing tackle present in or around the nest and 18.5% of all nestling injuries and 28.6% of all documented nestling mortalities were directly related to fishing tackle exposure. In conclusion, our study suggests that the risk of lead exposure from recreational fishing tackle is low even in a highly utilized recreational fishery, but risk does exist from fishing tackle does through entanglement and injury.

WP282 Screening for Genotoxic Agents with the DT40 Immortalized Avian Cell Line

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We regularly use toxicogenomics in primary avian cell cultures to screen and prioritize environmental pollutants of potential concern to wild bird species. However, primary cells are limited in their ability to identify agents that can cause genomic damage. This is largely because primary cells undergo very little DNA replication and cell division, which are two cellular functions generally required for most genotoxic modes of action. In contrast, the chicken B-lymphocyte DT40 cell line is an immortalized avian cell line that has been used increasingly in genotoxicity testing due, in part, to its very rapid growth rate. Here we incorporated the DT40 cell line into our in vitro toxicogenomics screening pipeline. DT40 cells were exposed to a variety of chemicals, including some known genotoxic agents. Gene expression profiles were determined using chicken ToxChip polymerase chain reaction (PCR) arrays which contain probes for genes in a variety of toxicologically relevant pathways, including cell cycle regulation and DNA repair. Chemicals with toxicogenomic profiles that suggested genotoxic activity were further assessed for genotoxicity using an in vitro flow cytometry micronucleus assay. Successful incorporation of the DT40 cell line into our in vitro toxicogenomics screening pipeline will provide the additional ability to detect genotoxic modes of action and significantly reduce the number of animals required for our chemical screening program. Future work will require the addition of metabolic enzymes into our screening assay, which are often required to activate the genotoxic activity of some chemicals. The sensitivity of this assay for genotoxicity may also be improved by using variants of the DT40 line with impaired DNA-repair capacity.

WP283 The effect of ingested aluminum on forage quality assessment and patch fidelity in honey bees (Apis mellifera)

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Aluminum concentrations are naturally variable in soil and may be increasingly bioavailable to organisms through mining activity and acid rain. Bioavailable aluminum can be absorbed into plant tissue and dispersed to other organisms through pollen or nectar collection and herbivory. Elevated bioavailable aluminum has been linked to inhibited growth in plants and malfunction of the cholinergic system in animals. Honey bees (Apis mellifera) can be used as a model organism to understand how aluminum affects the cholinergic system. Consumption of aluminum from floral nectar and pollen food resources may inhibit acetylcholinesterase activity resulting in disorientation, delayed learning and spasms. Preliminary data shows naturally occurring concentrations of aluminum may minimally shorten lifespan, however, how aluminum affects behavior and forage assessment and fidelity is not yet known. We will present behavior data of Apis mellifera caucasia foraging on an artificial flower patch with color-dependent sugar concentrations to determine color fidelity before and after aluminum treatment and when fed sucrose alone. This allows us to understand how forage quality assessment is affected by potentially neurotoxic aluminum. If aluminum is affecting foraging decisions is may be reducing hive survival and may similarly contribute to cholinergic malfunctioning in vertebrates such as is seen in neurodegenerative disorders.

WP284 The Unforeseen Challenges of Pollinator Toxicity Test Matrices

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The USEPA has indicated an intention to codify requirements for toxicity data from laboratory tests with honey bees (Apis mellifera) as part of Tier 1 requirements for the registration of pesticides under the Federal Insecticide, Rodenticide, and Fungicide Act (FIFRA). The five required honey bee Tier 1 toxicity tests are adult acute oral, adult acute contact, larval acute oral, adult chronic oral, and larval chronic oral. The adult oral and contact studies have been conducted under finalized guidelines for a number of years. A recently finalized guideline exists for the larval acute oral test, but only draft guidelines are available for the newer chronic adult oral and chronic larval studies. Experience with the conduct of these draft guideline studies is still limited. One of the more challenging aspects of the testing has been the analytical confirmation of the dosed diets to be consumed by the adult or larval bees... The dosing matrices (50% sucrose solution for adult bees and royal jelly diet for larval bees) are quite different than most matrices used in ecotoxicological testing and, as such, prove to be quite challenging for chemists and ecotoxicologists alike. In addition to the uniqueness of these diet matrices, there are complications with the unusually high dose levels often needed for test materials with limited toxicity to bees. Working with these liquid colloidal diets has led to unforeseen analytical challenges sometimes producing unreliable analytical results. We will discuss a procedure we have developed to assess the homogeneity and reproducibility of dosed diets used in honey bee toxicity tests. This procedure is intended to demonstrate dose confirmation of adult or larval honey bees during testing.

WP285 Toxaphene causes deformities and mitochondrial dysfunction in developing zebrafish embryos

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Toxaphene is a restricted use pesticide that is a mixture of over 670 different chemicals produced by reacting chlorine gas with camphene. This pesticide was used heavily in the Lake Apopka region in Florida for agriculture, and today it remains elevated in the soil despite being banned for more than 50 years. Toxaphene has been reported to elicit adverse effects in both mammals and fish, and increased cancer risk has been demonstrated in laboratory rodents. The objective of this study was to determine the effects of toxaphene exposure on zebrafish early life stages, a model fish species widely used for developmental toxicity research. Zebrafish were exposed to 0.011-11.1 µg/L from 6 hours post fertilization (hpf) to 96 hpf. Significant mortality was observed in embryos exposed to >1.11 µg toxaphene/L. Deformities were noted at >0.11 µg/L doses, and included edema as well as spinal and cranial deformities. As energy production is important for development and may be related to the observed deformities, mitochondrial bioenergetics of 24 hour embryos were assessed following exposure. ZF embryos were exposed to 0, 11.1, or 111 µg toxaphene/mL (0.5% v/v DMSO) for 24 hours starting at the blastula stage (~3 hpf). Oligomycin, the uncoupler FCCP, and antimycin A were used to challenge the mitochondria in a stress test. Twenty-four hour old embryos treated with 11.1 and 111.1 µg/L showed lower basal respiration and lower ATP-dependent respiration compared to vehicle and water controls, suggesting that the capacity of the mitochondria to produce ATP was compromised following exposure to toxaphene. Future efforts are focused on the mechanisms of altered bioenergetics following toxaphene exposure, specifically on transcripts that are associated with mitochondrial dysfunction. Altered bioenergetics in early development can result in delayed hatch and compromised survival of wild species and studies are underway that are investigating wild, indigenous species in Florida.

WP286 Toxicity of polychlorinated biphenyls and N-phenyl-1-naphthylamine in juvenile turtle Chelydra serpentina

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Polychlorinated biphenyls (PCBs) as well as substituted phenylamines (SPAs) antioxidant are two chemical groups that have been used in multiple Canadian industrial processes. Despite the ban of PCB production in North America in 1977, they are still ubiquitously found in the environment. Previous studies with mammals, birds, amphibians and fish have shown PCBs to be neurotoxic, genotoxic, teratogenic, and they have been classified as endocrine disruptors. In contrast, SPAs, specifically N-phenyl-1-naphthylamine (PANA), have received very little attention despite their current use in Canada and their expected aquatic and environmental releases. There is a research gap regarding the effects of PCBs in reptiles and PANA in wildlife; therefore, snapping turtle (Chelydra serpentina) was studied due to its importance as an environmental indicator. The first experiment was conducted using food pellets spiked at an environmentally relevant concentration of the PCB mixture Aroclor 1254 (A1254) to model bioaccumulation and depuration of PCBs in the turtle's liver. Turtles were fed food contaminated with 500 ng/g A1254 for 31 days followed by clean food for 50 days. No differences were observed between the control and treated animals. This suggests that juvenile turtles exposed to 500 ng/g PCBs eliminated PCBs at a fast enough rate as to avoid bioaccumulation. Two additional dose-response experiments were performed using A1254 and PANA spiked food to determine hepatic toxicity and bioaccumulation in juvenile C. serpentina (0-12,500 ng/g and 0-10,000 ng/g, respectively). An increase in cyp1a was observed when exposed to the highest dose of A1254. PCBs are known endocrine disruptors, but although non-significant increasing trends were observed for both thyroid receptors α and β, no changes were found for aromatase or estrogen and androgen receptors, indicating that C. serpentina is less sensitive to PCB endocrine disruption than other species. Similarly to PCBs, a significant increase in cyp1a mRNA expression was also observed when exposed to the highest dose of PANA. Additionally, a suite of oxidative or cellular stress genes was studied for both PCB and PANA exposed animals, but none of the genes were altered by any treatments. Overall, this study has demonstrated the toxicity of a persistent and an emergent contaminant, which will help monitor and predict health risks associated with environmental contamination for C. serpentina populations.

WP287 Toxicological effects of Triclosan (TCS) and Triclocarban (TCC) to a model organism the nematode Caenorhabditis elegans

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Triclosan (TCS) and triclocarban (TCC) are antimicrobials that are widely used in soaps, plastics, clothing, and other household products, and have been detected in surface waters including the Great Lakes. Yet the potential environmental health implications of these compounds have not been well understood. Recent studies suggest that they may have adverse effect to aquatic life due to their potential endocrine-disrupting properties. Here we assessed the potential toxicological effects of TCS and TCC using a model organism the nematode Caenorhabditis elegans (C. elegans) using endpoints from organismal to molecular levels, including lethality, reproduction, lifespan, hatching rate, germline toxicity, and oxidative stress. Larval or young adult worms were exposed to environmentally relevant concentrations of TCS and TCC and examined using above-mentioned endpoints. Both TCS and TCC showed significant acute toxicity to C. elegans, with 24-h LC50s of 4.1 mg/L and 0.35 mg/L, respectively. In terms of sublethal effects, exposure to 1 mg/L of TCS and 0.01 mg/L of TCC, caused a 32% and 34% reduction in the number of offspring, respectively. Hatching time was extended by 6 hours, from 9 hours to 15 hours, or by 67% for both TCS (2 mg/L) and TCC (0.1 mg/L) at 20°C. Lifespan was decreased on average by 3.7 days and 3.2 days for TCS (2 mg/L) and TCC (0.1 mg/L), respectively. To understand the potential mechanism of observed toxicity on reproduction, a transgenic strain of the worm containing the fluorescent reporter Pxol-1::GFP was used to detect germline disruption by these compounds. Exposure to concentrations as low as 1 mg/L of TCS and 0.01 mg/L of TCC resulted in disruption in an average of 3.2 and 4.1 eggs per worm, respectively. Using the daf-16::GFP reporter system, significant oxidative stress was found in worms exposed to both compounds at concentrations as low as 1 mg/L of TCS and 0.01 mg/L of TCC. Collectively, these findings suggest that TCS and TCC may pose significant health risks to aquatic organisms. Our future work will focus on understanding the underlying mechanisms of these observed toxic effects as well as examining how environmental factors may affect such toxicity.

WP288 Trophic transfer of mercury in a terrestrial food chain from a contaminated floodplain

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Mercury (Hg), especially when transformed by environmental microbes into methylmercury (MeHg), is one of the only metals known to biomagnify in food chains, leading to elevated concentrations in higher trophic level animals. Because human exposure to Hg is through the consumption of contaminated fish, Hg biomagnification in aquatic food chains has been well characterized. However, recent studies have highlighted that Hg bioaccumulation in terrestrial food chains can be significant. Ecological risks from Hg are related to environmental concentrations, but perhaps more than any other contaminant, the magnitude of Hg bioaccumulation and risk is defined by a variety of site-specific environmental factors. East Fork Poplar Creek (EFPC), a Hg-contaminated stream in Oak Ridge, TN, provides a useful case study for evaluating the role of Hg-contaminated floodplain soil in a terrestrial food chain. The Carolina wren and the short-tailed shrew were targeted as endpoint receptors for evaluating the potential risk to wildlife from food chain exposure to Hg in the EFPC floodplain. Consequently, our goal was to characterize the bioavailability of Hg and MeHg in prey items of these two ecological receptor species. We collected soil, leaf litter, and invertebrates from different feeding guilds: herbivores (leaf hoppers), detritivores (earthworms and wood lice), and predators (wolf spiders). Samples were taken from randomly selected plots of varying distance from the stream and corresponding to a gradient of soil Hg concentrations. We analyzed samples for Hg and MeHg to calculate site specific bioaccumulation factors (BAFs) and stable isotopes ($\delta^{13}C$ and $\delta^{15}N$) to infer trophic structure of the invertebrate groups. Differences in total Hg, MeHg, % MeHg and BAFs among the invertebrate prey groups were greater than those among sampling sites. Total Hg concentrations were significantly different among all invertebrate prey groups with Hg and BAFs in worms > woodlice > spiders > leaf hoppers, while MeHg concentrations in spiders = woodlice > worms > leaf hoppers. Methylmercury concentrations in invertebrates were strongly correlated with trophic position but were not related to Hg concentrations in soil. Our results are relevant to the broader understanding of Hg bioaccumulation in terrestrial food chains, and to remediation decisions at this and other contaminated sites.

WP289 Urbanization effects at different biological organization levels of estuarine polychaete Laeonereis acuta

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Estuaries are ecologically important environments for many species, represent the boundary between fresh and sea water, the ideal environment where successfully perform their entire life cycle. Among polychaetes species the Nereididae Laeonereis acuta is widely spread in the South America Atlantic estuaries and sheltered areas, from 2°S to 42°S. The aim of this study was to assess the effects of diffuse pollution caused by urbanization in estuaries at different biological organization levels of the polychaete L. acuta. The study was conducted from August to September in 2014 at ten estuaries located between coordinates 25º5'S – 48º3'W and 27º5'S – 48º4'W. The effects of urbanization on different levels of biological organization of the polychaete were assessed using: i) the frequency of micronuclei, at subcellular level; ii) the body size and biomass, at individual level; and iii) the production-to-biomass ratio (P/B), at populational level. The biomass values of sampled individuals from each site were used to calculate empirically the production-to-biomass ratio of the population, uzing the formula adapted from model of Brey (2012). The determination of total nitrogen, total phosphorus and total phenols was performed by spectrophotometry according to Standard Methods (APHA, 1999).The aluminum, cadmium, chromium, copper and lead contents in sediments were obtained by inductively coupled plasma mass spectrometry. The spatial distribution of L. acuta samples was distinct between the urbanized and non-urbanized estuaries. Samples from urbanized estuaries were characterized by higher concentrations of metals (lead, cadmium, and chrome) and dissolved nutrients (phosphorus and nitrogen). Otherwise, biological samples of non-urbanized estuaries were more related with sandy sediments and well sorted grains (low variance). The analysis of variances of environmental variables showed significant higher values for aluminum, cadmium, lead and sorting in sediment as well as nitrogen in water from urbanized estuaries than from non-urbanized ones. The lower levels of biological organization (frequency of micronuclei at subcellular level; body size and biomass at individual level) showed higher values in urbanized than in non-urbanized estuaries. Contrarily, the higher level of biological organization assessed, P/B ratio of population, was significantly higher in non-urbanized estuaries than in urbanized ones.

WP290 Use of an In Vitro Protein Binding Assay to Characterize Interactions of OP Triesters and Metabolites with Thyroxine and Human Transthyretin

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Organophosphate triester flame retardants (OPFRs) have been applied to plastics, foams and textiles for decades, and production of these additive flame retardants has increased substantially since the phase-out of polybrominated diphenyl ethers (PBDEs). Due to leaching, various OPFRs have been found in both indoor and outdoor environments leading to human and wildlife exposure. Recent studies have shown that the OPFR triphenyl phosphate (TPHP) is largely metabolized to hydroxylated metabolites, but also dealkylated to organophosphate diesters e.g. in vitro using human and herring gull liver microsomal assays as well as in vitro in chicken embryonic hepatocyte assay. Toxicological properties of OPFRs are not well understood to-date, though increasing evidence suggests possible effects on the thyroid system. Perturbation of thyroid hormone transport is considered to be one mechanism of action that may affect thyroid function, and this is a toxicological concern with PBDEs and especially hydroxy-BDE metabolite compounds. As such, the objectives of this study were to a) optimize an in vitro competitive protein binding assay that uses thyroxine (T4) as the natural ligand to thyroid hormone binding protein transthyretin (TTR, from human plasma), and b) apply this assay to investigate the abilities of select OPFRs identified in environmental biota, and diester and/or hydroxylated metabolites for each, to competitively displace T4 from TTR. The present method employs size exclusion chromatography, using pre-packed polyacrylamide gel filters, to separate free and protein-bound radiolabeled T4 across a series of concentrations of each organophosphate competitor. The characteristics of these interactions will be presented herein.

WP291 Wildlife Mortality Caused by Bromethalin: Implications for Incident Investigation and Risk Assessment

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Recent regulatory restrictions of second generation anticoagulant rodenticides have led to an increased use of bromethalin in rodenticide bait products, and a potential for increased exposure to non-target wildlife. Bromethalin poisoning is quite distinct from anticoagulant poisoning, with abnormal behavioral and coordination impairment caused by neurologic dysfunction being the prominent signs of toxicity, rather than the uncontrolled hemorrhage caused by anticoagulant rodenticides. Recent reported incidents of wildlife mortality attributed to primary bromethalin poisoning have involved omnivorous mammalian species common to urban and suburban areas, including gray squirrels, raccoons, and striped skunks. Two gray fox poisonings have been reported; however, it is unknown if the foxes died from primary or secondary exposure. Notably, no mortality incidents involving raptors have been reported. Observation of granular green or turquoise material in the colon or in fecal matter, indicating possible ingestion of bromethalin bait, has been useful as a diagnostic indicator of bromethalin poisoning. Initial suspicion of bromethalin poisoning were confirmed by detection of the primary metabolite, desmethylbromethalin, in adipose and/or brain tissue, or the detection of bromethalin in the contents of the gastrointestinal tract. The co-occurrence of anticoagulant rodenticides in liver tissue has often complicated the diagnosis. Frequencies and patterns of wildlife incidents suggest that bromethalin poses a risk to non-target mammals from primary exposure, whereas risk from secondary exposure appears to be less than for anticoagulant rodenticides. Possible reasons for the reduced secondary risk are discussed.

WP292 Carolina Wren Revisited: The Challenges of Field Study

B. Henry, J. Murauskas, Anchor QEA

Field studies of contaminant impacts on avian nest success present numerous challenges for quantitative assessment. We analyzed the study design and data used to model a predictive relationship between blood mercury (Hg) concentrations and reduction in nest success of Carolina wren (Thryothorus ludovicianus) (Jackson et al. 2011), which has been considered for use in assessments of risk and natural resource damages. Our analysis indicated that study design and data interpretation were insufficient for predicting reduction in nest success at specific blood Hg concentrations. The reference site selection process was not documented, the number of samples was small (i.e., only two and four abandoned nests at reference and contaminated sites, respectively, in the 2010 dataset of 40 nests used in the predictive model), and the mixed use of artificial (box and tube) and natural nests (with artificial nests disproportionately used in contaminated sites in 2010) confounded interpretation. We calculated 30-day nest success and used the mathematical model MARK, similar to Jackson et al.'s use of MCestimate, to evaluate various statistical models to explain observations. For the pooled 2007 to 2009 dataset where all but one cavity was artificial (and therefore cavity type was controlled), 30-day nest success based on abandoned nests was the same at reference and contaminated sites. In 2010, 30-day nest success based on abandoned nests in the reference sites was higher than in the contaminated sites and higher than both in 2007-2009, however the reference sites had twice as many natural cavities as the contaminated sites. Our MARK results based on abandoned nests indicated that cavity type was an important covariate, along with location. While nest success data and mathematical models have potential for assessing contaminant impacts, robust studies require extensive data collection and accounting for multiple factors other than contaminants that impact nest success and, perhaps more importantly, fecundity. In addition, variable results among years indicate the need for multi-year studies. Mathematical models are powerful; however, conclusions are ultimately limited by the quality and quantity of observations. The Jackson et al. predictive relationship between blood Hg and reduction in nest success is compromised by its study design and small dataset, and therefore should not be used for quantitative estimation of risk, injury, or service loss to songbirds.

WP293 The Effect of Scaling Factors on Terrestrial Organism Pesticide Risk Assessments

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EPA relies on the T-REX model for conducting pesticide risk assessments on birds and mammals, and often extrapolates the results for birds to reptiles and terrestrial-phase amphibians. This model focuses on potential risks to birds and mammals through ingestion of feed items containing pesticide residues. T-REX contains a set of default feed item categories, which include grasses, broadleaf plants, arthropods, fruits, and seeds. The model estimates potential acute and chronic risks to three size classes of birds (20 g, 100 g, 1000 g) and mammals (15 g, 35 g, 1000 g). T-REX also includes default scaling factors that adjust the toxicity endpoints for birds and mammals based on the weight of the tested animals compared to the three weight classes of birds and mammals evaluated by the model. For avian assessments the scaling factor adjusts the toxicity endpoints so that endpoints for smaller birds are lower than those for larger birds. In contrast, for mammals the scaling factor adjusts the toxicity endpoints so that endpoints for larger mammals are lower than those for smaller mammals. The overall result of using these scaling factors is to significantly change the toxicity endpoints from those determined in standard toxicity testing, which impacts conclusions concerning potential risks to different size birds and mammals, including endangered species. In contrast to these estimated toxicity endpoints, actual testing of animals of similar sizes to the sizes evaluated by T-REX often results in very different actual toxicity values. Examples comparing T-REX estimated toxicity values to actual toxicity values for several pesticides will be provided, along with discussion of the impact of these discrepancies on risk assessment conclusions. Impacts on risk conclusions concerning endangered species are much more likely because the bar for concluding possible effects on endangered species is much lower than for non-endangered species.

Aquatic Toxicology and Ecology – Poster Only – Part 3

WP294 Exploring mechanisms of organophosphate resistance in natural populations of the crustacean Hyalella azteca

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Hyalella azteca are freshwater, epibenthic amphipods widely used in environmental toxicology to study the impacts of human activities including the consequences of pest management. In recent years, resistance to pesticides has been documented not only in insect pests but also non-target organisms including H. azteca. One such group of pesticides facing resistance is the organophosphate (OP) class, which targets and inhibits the neurotransmission enzyme acetylcholinesterase. While most wild populations of H. azteca, including the strain commonly used for toxicity testing, are susceptible to organophosphates, select wild populations of H. azteca are 5-1000 fold more tolerant to OPs compared to background sensitivity. The emergence of resistance in a non-target organism reveals environmental implications of pesticide application beyond their immediate insect target; therefore, there is a critical need to better understand the mechanisms and possible ecological consequences of organophosphate resistance. Our study investigated the genetic basis of OP resistance in H. azteca by examining the acetylcholinesterase (Ace) gene, the target site of OP insecticides, in both susceptible and resistant populations. In particular, a single amino acid substitution, G119S in Ace has been highly correlated with OP resistance in target pests. We found that although lab populations did not harbor this mutation, the G119S mutation was present with up to a 40% frequency in select resistant populations. In addition, through protein modeling we showed that this substitution alters the active site providing further support for its role in OP resistance. However, the presence of the mutation is not sufficient to explain differences in the sensitivity across resistant populations. As an alternative, and likely complementary mechanism of resistance, we also dove into the newly assembled H. azteca genome to identify and annotate genes involved in OP metabolism. Increases in gene expression of metabolic enzymes such as glutathione-S-transferases (GSTs) may provide further protection against OP insecticide toxicity. We annotated a total of 28 GSTs, which is similar to the expected number in crustaceans, providing confidence that we identified a nearly complete set of GSTs in H. azteca. Through the further investigation of Ace mutations along with expression levels of GSTs, we expect to fully characterize the mechanisms of OP resistance in natural populations of H. azteca.

WP295 Assessing the Developmental Neurotoxicity of 27 Organophosphorus Pesticides Using a Zebrafish Behavioral Assay

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The U.S. Environmental Protection Agency is evaluating methods to screen and prioritize organophosphorus pesticides for developmental neurotoxicity. As such, we are exploring a behavioral testing paradigm that can assess the effects of sublethal and subteratogenic concentrations of developmental neurotoxicants on zebrafish (Danio rerio). This in vivo assay quantifies the locomotor response to light stimuli under tandem light and dark conditions in a 96-well plate using a video tracking system on 6 day post fertilization zebrafish larvae. Each of twenty-seven organophosphorus pesticides was tested for their developmental neurotoxic potential by exposing zebrafish embryos/larvae to the pesticide at several concentrations (≤ 100 μM nominal concentration) during the first five days of development, followed by 24 hours of depuration and then behavioral testing. Approximately 22% of the chemicals (Acephate, Dichlorvos, Diazoxon, Bensulide,Tribufos, Tebupirimfos) did not produce any behavioral changes after developmental exposure, while many (Malaoxon Fosthiazate, Dimethoate, Dicrotophos, Ethoprop, Malathion, Naled, Diazinon, Methamidophos, Terbufos, Trichlorfon, Phorate, Pirimiphos-methyl, Profenofos, Z-Tetrachlorvinphos, Chlorpyrifos, Coumaphos, Phosmet, Omethoate) produced changes in swimming activity. Some of the latter group (Diazinon, Omethoate, Pirimiphos-methyl, Profenofos, Terbuphos) were particularly potent for developmental neurotoxicity, showing behavioral alterations at doses 2 to 3 orders of magnitude below the developmental concentrations that produced death or dysmorphology. These results indicate that some of these organophosphorus pesticides may elicit neurodevelopmental toxic effects in zebrafish at concentrations below those that produce lethality or dysmorphology. This abstract may not necessarily reflect official Agency policy.

WP296 An assay for quantitative detection of reactive toxicity in whole zebrafish embryos – case of two pesticides

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Reactive toxicity is a first line response of cells upon exposure to toxic substances and part of the process that leads to cytotoxicity of contaminants. Detection of reactive toxicity in complex organisms can help to identify cell stress way before effects become visible on an organ or organism level. This allows for an early and sensitive identification of adverse effects. A zebrafish embryo assay that uses a fluorescent dye for microscopic detection of glutathion was adapted to a 96-well plate format to allow for quantification of reactive toxicity through fluorescence measurement with a multiwell plate reader. The assay was initially developed using tert-butyl hydroperoxide (t-BHP) as a reference substance, and then benchmark tested against the two pesticides diazinon and diuron. Long and short-term exposure scenarios and different protocols for the fluorescent staining were used. Hatched larvae (96 hpf) were then anesthetized, transferred to 96-well plates with conical wells, and fluorescence measured using a tailored detection method for a multiwell plate reader. Embryos were also inspected under a fluorescence microscope to qualitatively record locations and intensities of reactive toxicity in the individual specimen. Prior to the assay, lethal and sublethal effects of the pesticides on zebrafish embryos had been determined in a common FET test. We found that the assay is capable of detecting reactive toxicity of diazinon and diuron at concentrations as low as 0.1 mg/L and 0.02 mg/L, respectively, using the long-term exposure scenario. For the short-term exposure LOECs were 2 mg/L for both pesticides. For the reader measurement we could not observe any difference in the data of repeated measurements after thorough shaking, while the position of the embryo in the well turned out to be crucial for microscopic inspection. The assay allows to detect effects on zebrafish embryos at an early stage and at a low level of exposure clearly before deleterious consequences on an organ or organism level become visible. Since the embryos are kept alive, subsequent investigations of, e.g., EROD or micronucleus induction or further enzymatic biomarkers are possible. We will use the assay to investigate the impact of reactive toxicity on mechanism-specific biomarkers in zebrafish embryos. This would allow to verify whether biomarker results are representing a specific mode of action, or are rather the consequence of decreasing cell fitness.

WP297 Assessment of the toxicity of dichlorvos on organisms of different trophic levels

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Dichlorvos (DDVP) is an organophosphate insecticide considered by the EPA as highly toxic. This compound is effective in controlling lepidopteran, mites and homeópteros. It is used to control pests in domestic animals and to eliminate exogenous parasites in salmon aquaculture farms. Because there are few studies of the effects of DDVP in aquatic organisms The objective of this study was to evaluate the toxicity of dichlorvos in organisms of different trophic levels: Cladocerans: Daphnia magna, Daphnia exilis, Daphnia pulex and Simocephalus mixtus. The ostracod Cypris sp. and fishes: juvenile charal (Chrirostoma jordani) and juvenile zebrafish (Danio rerio). Furthermore their sublethal effects were evaluated by means of assessment of four biomarkers (growth rate, O:N index, lipoperoxidation and inhibition of acetylcholinesterase enzyme). Acute bioassays were performed, the organisms were exposed to 6 pesticide concentrations to determine the LC_{50}. Subsequently tests with duration of 15 days were made where the organisms were exposed to a sublethal concentration (LC_{10}), for assessment of 4 biomarkers. In the results it was evident that the cladoceran Daphnia exilis and juvenile Chirostoma jordani are more sensitive to DDVP compared to other species. The O:N index had values below 9 fact indicates that organisms were in a high degree of stress. Growth rates of intoxicated organisms were between 19 to 49% lower than those observed in the control group. The degree of lipid peroxidation was increased up to 200% in the exposed organism. A decrease in AChE activity was observed in cladóceros between 22 and 45% and fish from 22 to 35%. The results of this study indicate that the effects of the pesticide DDVP in organisms under study are likely irreversible.

WP298 Comparison of the transcriptional impact of chlorpyrifos and permethrin onolfaction in zebrafish larvae and adults

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A variety of essential activities and behaviors of fish, for instance food detection, kin recognition, homing, the predator response and mating habits, depend on olfaction. Consequently, an impairment of the olfactory system can affect both individual fish and whole populations. Despite the importance of the olfactory system for fish, only few studies investigating an impairment of the latter were published and little is known about underlying mechanism. The olfactory epithelium (OE) is locatedin two pits at the dorsal part of the snout. Through these pits the surrounding water is constantly flowing. The olfactory receptor neurons (ORNs), which are responsible for odorant detection, are situated within the OE and only separated from the environment by a thin layer of mucous. In such an exposed situation dissolved xenobiotics can interact with them as easily as natural odorants do. The olfactory system in zebrafish larvae is present albeit not fully developed yet. The number of neurons in the olfactory bulb (OB), for instance, counts 5 % of the amount of neurons in the OB of adult zebrafish. Previous studies have shown that several metals and pesticides are able to influence the olfactory system of fish in environmentally relevant concentrations. However, underlying mechanisms predominantly remain unidentified. The current study compared the effects of short term exposures (3 and 24 h) to sublethal concentrations of the commonly used pesticides chlorpyrifos and permethrin on the gene expression of marker proteins for the three different types of ORNs (olfactory marker protein B (ompb), transient receptor potential cation channel, subfamily C, member 2 (trpc2), s100 protein (s100z) and enzymes with protective function during oxidative stress (peroxiredoxin-1 (prdx1) and hemeoxygenase-1 (hmox1)) in both zebrafish larvae and adults. Thereby, information about the suitability of zebrafish larvae as an alternative test model for olfactory toxicity is gathered. Neither permethrin nor chlorpyrifos induced the gene expression of hmox1 and prdx. 24 h exposure to chlorpyrifos led to a concentration dependent slight decrease in the expression of ompb and trpc2. Following 24 h exposure to permethrin a minor decline of the gene expression of ompb and s100z was detected. The collection of the gene expression data of adult zebrafish is still in progress, but will be ready for presentation until the start of the conference.

WP299 The effect of chlorpyrifos on salinity acclimation of rainbow trout: Serum hormones and gene expression changes in liver, gill and olfactory rosettes

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As a part of their unique life cycle, most salmonids undergo transition from freshwater to saltwater, requiring various adjustments in metabolism, osmoregulation and ion regulation. The hypersaline acclimation of salmonids can be affected by pesticides during downstream migration of fish to the estuary. The present study aims to determine how toxicity of chlorpyrifos (CPF) impacts hypersaline acclimation of Steelhead/rainbow trout (Oncorhynchus mikiss). We exposed fish (52±5 g, 4 fish in each 4 replicate 20L glass tank) to 0, 20, 40, 80, 160 µg/L of CPF for 7 days, and then to hypersalinity (12ppt) for another 7 days. Blood or tissues (liver, gill and rosette) were collected after exposure. Mortality, levels of cortisol, T3 and T4 in serum, and expression of genes involved in detoxification, ion transportation and neural signal transduction were measured at the 7th day (CPF exposure) and 14th day (salinity) exposure. CPF exposure did not significantly alter cortisol levels, but fish exposed to hypersaline conditions showed higher cortisol levels than freshwater control groups. Serum thyroid hormones T3 and T4 were also increased after CPF exposure as well as during the salinity acclimation. Hepatic mRNA of Glutathione-S-Transferse pi (GST) was increased up to two fold following exposure to CPF and a similar trend was observed after hypersaline exposure. Expression of Na+/K+ATPase α1 mRNA in the gills was increased 4 fold during salinity exposure but was unchanged by CPF. These results indicate CPF may alter endocrine pathways that influence osmoregulation and growth in fish as they reach hypersaline water.

WP300 Exposure to a Common Pesticide, Butachlor, alters Tadpole Morphology

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Butachlor is the most common herbicide used by rice farmers in the Philippines and throughout Southeast Asia. At concentrations much lower than the recommended application rate of 4.8 mg/l, Butachlor exposure has been linked to genotoxicity, neurotoxicity, carcinogenesis, and endocrine disruption in insects and amphibians. Rice fields provide substitute wetland habitat for several amphibian species that can be used as sentinels of farmer exposure risks. Amphibians represent valuable bio-indicators because they have the same general endocrine glands and hormones that control growth, development, reproduction, and other physiological responses in humans. Our experiment tested the effects of butachlor and interspecific competition as a second stressor on the growth and development of two species of frogs, Fejervarya vittigera (native) and Rhinella marina (invasive). Because of the success of the invasive species in our study population, we hypothesized that R. marina would be less affected by butachlor than F. vittigera. We conducted both geometric morphometric and traditional morphometric analysis using TSP DIG 264, MorphoJ, GeomoRph, and Image J. We measured total body, snout vent, tail, tail width, and hind limb length as well as general tadpole shape using landmark and semi-landmark coordinates after a 15-day exposure. Our results demonstrated that overall morphology and development in F. vittigera was slowed in butachlor and competition treatments whereas in R. marina development was much faster in butachlor and competition treatments. The results indicate that butachlor affects morphology and development in a species-specific fashion. Because amphibian development is under control of both thyroid hormone and stress hormones, we will investigate the underlining endocrine mechanisms that may influence development in these species when exposed to butachlor.

WP301 Oxidative Damage to DNA and Lipidic Peroxidation in Oreochromis niloticus exposed to the fipronil insecticide during periods of hypoxia

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Fipronil is a pesticide used in sugarcane, persistent in soil and has been widely detected in aquatic environments. Its presence in water compartments can influence and promote changes in physiological processes of fish, causing oxidative stress, which can be demonstrated by increased lipid peroxidation in different organs, which is measured by the levels of malondialdehyde (MDA). Whereas, adverse effects on the environment such as the condition of hypoxia, can negatively influence in this process and cause changes in DNA integrity, we propose to evaluate the influence of exposure to a commercial formulation of fipronil (Regent®800WG) on parameters of lipid peroxidation in gills of Oreochromis niloticus under hypoxic and its effect on DNA. The fishes used in this study were exposed for 3 and 8 hours to fipronil in two concentrations (0,1 e 0,5 µg L^{-1}), in the presence and absence of hypoxia. The low oxygen concentrations in the water were maintained by bubbling with gaseous nitrogen. For each exposure time it was maintained a negative control. After each exposure times, the animals were collected and then evaluated the degree of lipid peroxidation in gills and oxidative damage to DNA by comet assay in fish erythrocytes. The results were submitted to the Kruskal-Wallis statistical test and post hoc Newman-Keuls and considered statistically significant difference with $p < 0.05$. The results indicated significant decrease in MDA levels in the gills of animals exposed to 0.5 ug L^{-1} concentrations compared to control, with the largest decrease in MDA levels observed in the groups exposed to hypoxia in combination with fipronil in the two concentrations tested. Some studies have shown that significant decreases in MDA levels upon exposure to different pollutants are related to the response to stimulation antioxidant. This effect could be observed in the groups exposed to hypoxia together with Fipronil, which showed a higher rate of oxidative damage to DNA, suggesting that, under hypoxia, the sites of electron transport chains are lower due to limited oxygen availability. So, there are more electrons to escape the fetters and add oxygen molecules, increasing the risk of tissue damage. These results indicate that Fipronil is capable of inducing cell damage in environmentally relevant concentrations, and their action can be compounded when coupled with environmental factors.

WP302 Evidence of DDT resuspension through dredging of a Midwest agricultural lake system

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Although the organochlorine dichlorodiphenyltrichloroethane, commonly referred to as DDT, was outlawed in select countries during the 1972 Stockholm Convention due to its long half-life and ability to sorb to substrate, DDT and daughter compounds can persist in the environment for approximately forty years. When sorbed to substrate, DDT does not pose a measurable threat, but once large quantities are disturbed by anthropogenic activities such as dredging, DDT could be released into the water column and recirculate through the ecosystem. Due to the lipophilic nature of DDT, resuspension and bioaccumulation in organisms readily consumed by humans may occur. This pilot study attempted to determine if DDT compounds were present at measurable levels in Storm Lake, a dredged 3,097 acre lake located in agricultural Iowa. Storm Lake averages a 20 foot depth with UTM coordinates being Zone 15T: (0318509E) (4723248N). The lake system was formed during the Wisconsin Glaciation period and is commonly used for recreation. Due to its relatively shallow depth, dredging began in Storm Lake in the early 2000's and continues to present time. This study worked with three substrate samples contributed by FYRA Engineering, Omaha, NE, which was contracted by the local Lake Improvement Commission to collect a series of 12 substrate samples for siltation analyses. The three samples used for this analysis were chosen based on when the site had been dredged. Site A had not been dredged, Site B was dredged in 2011, and Site C was dredged in 2015. To determine concentrations of DDT compounds in the samples, an external calibration curve was created through analysis of 0 ppm, 0.1 ppm, 0.2 ppm, 0.25 ppm, 0.5 ppm, and 1 ppm concentration solutions of DDT Mix Neat from Ultra Scientific in acetonitrile on an Agilent 7890A/5975 GC/MS. The GC was equipped with an HP5MS column, and Helium was used as the carrier gas at a flow rate of 1.0 mL/min. A range of m/z 50–500 was scanned to confirm the retention times of the analytes. The calibration curve related the DDT Mix concentrations in ppm to the abundance peak area at a retention time of 17.23 minutes for DDD and 18.61 minutes for DDT. Results indicated that DDT levels in selected Storm Lake sites were non-detectable, but this study plans to continue research investigating DDT bioaccumulation and metabolomics at all 12 sites using planarians as a biological model.

WP303 Effect of flow on bioaccumulation of DDT and other organochlorine pesticides in an apex aquatic predator from Kruger National Park, South Africa

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With the top gross domestic product in Africa, South Africa is known to have a high pesticide usage, which includes the highly persistent and banned group of organochlorine pesticides (OCPs). South Africa is also one of few countries to still actively spray DDT as malaria vector control. The aim of the study was therefor to determine the degree to which aquatic biota in selected rivers of the world renowned Kruger National Park (KNP) are exposed to OCPs used in the catchments outside the KNP. Tigerfish, Hydrocynus vittatus, an economically important apex predator, was selected as bioindicator for this study. Fish were sampled from the KNP sections of the Luvuvhu, Letaba and Olifants Rivers during the high and low flow periods from 2010 to 2012 and OCPs were determined in muscle tissue using GC-ECD techniques. Significant flow related and spatial (different rivers) OCP bioaccumulation was observed. Tigerfish from the Luvuvhu River displayed the highest OCP bioaccumulation. Concentrations of the majority of the OCPs including the DDTs were the highest levels ever recorded from South African freshwater systems and in many cases the concentrations were higher than most contaminated areas from around the world. The concentrations found in

H. vittatus muscle also exceeded maximum residue levels in edible fat as set by the European Union. This is of concern when managing the water resources of the conservation area since the contaminants enter the park from outside the borders and pose potential risks to the mandated conservation of aquatic biota within the KNP.

WP304 Effects of Bifenthrin on the Estrogenic and Dopaminergic Pathways in Embryos and Juveniles of Zebrafish (Danio Rerio)

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Bifenthrin (BF) is a pyrethroid pesticide widely used in urban and agricultural applications. Previous studies in fish have shown that environmentally relevant concentrations of BF can affect the endocrine system by leading to the over production of 17b-estradiol (E2) and altering the expression of dopaminergic pathway components in the central nervous system in fish. Dopaminergic neurons regulate luteinizing hormone (LH) and follicle stimulating hormone (FSH), which control E2 biosynthesis, suggesting that BF may disrupt the hypothalamic-pituitary-gonad (HPG) axis. The hypothesis that BF impairs this pathway was tested in embryonic and one-month old juvenile zebrafish (Danio rerio). At 3 hours post fertilization (hpf), and one month of development, fish were exposed to 0.15 and 1.5 ppb BF for 96 hours. The relative levels of transcripts related to the dopaminergic and the HPG axis (tyrosine hydroxylase (TH), dopamine receptor 2A (DR2A), dopamine active transporter (DAT), sodium/potassium ATPase (NA/K ATPase), LHb, FSHb, and vitellogenin (VTG)) were investigated by qRT-PCR. Levels of E2 were measured by ELISA. Dopamine and its metabolites (3, 4-dihydroxyphenylacetic acid and homovanillic acid) concentrations were evaluated by LC-MS/MS. Preliminary results show significant decreases of TH, NA/K ATPase, and VTG transcripts in zebrafish embryos, and a significant increase in the expression of TH and DAT in zebrafish juveniles. Estradiol levels were significantly decreased in embryos. These results show a possible anti-estrogenic effect of BF in embryos, and estrogenicity in juveniles. Studies on dopamine concentrations are ongoing. Further analysis of differentially expressed genes coupled with endocrine responses can help assess potential toxic and sub lethal effects of BF using zebrafish as an animal model.

WP305 Effects of Bifenthrin on Sex Differentiation in Japanese Medaka (Oryzias latipes)

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Bifenthrin (BF) is a pyrethroid pesticide widely used in urban and agricultural applications. In vitro studies have indicated anti-estrogenic activity in this compound; however, in fish estrogenic activity has been observed. The Japanese Medaka (Oryzias latipes) is a unique model due to early life stage susceptibility to phenotypic sex reversal upon exposure to estrogenic compounds. Moreover, it is well known that this fish express the male-determining DMY gene,an easy way of verifying genotypic sex. The hypothesis that BF impairs sex determination was tested in larval and embryonic Japanese Medaka. Fish were exposed, in vivo, to environmentally relevant concentrations of BF (0.15ppb and 1.5ppb), a single concentration (0.5µM) of an E2 receptor antagonist (ICI 182780), and an agonist (E2). Separate exposures were implemented at 2 hours post fertilization (hpf) for 10 days and 7 days post hatch (dph) for one month to simulate environmentally relevant exposures at sensitive stages of embryonic and larval development, respectively. Phenotypic sex, secondary sexual characteristics (SSC) and genotypic sex were investigated at sexual maturity. Preliminary results show trends of increased feminine SSC scores in Japanese Medaka exposed to BF at the embryonic stage ($p = 0.083$), but not at the larval stage ($p = 0.924$). Mann-Whitney U tests indicated differences between 1.5ppb BF and ICI 182780 with increased feminization in BF ($p = 0.02$) following embryonic exposure. In addition, Mann-Whitney U tests also indicated significant differences between 0.15ppb and 1.5ppb BF ($p = 0.02$) with higher feminization in the 1.5ppb BF embryonic exposure. Although, a trend toward masculinisation in sex ratios was observed for ICI 182780, reductions in male fish percentages for 0.15ppb and 1.5ppb BF were observed. Ongoing studies will compare phenotypic values of SSC with DMY expression in the same individuals. These results show a possible estrogenic effect of BF in embryos, affecting sex determination.

WP306 Anti-predator responses of freshwater snails following chronic exposure to atrazine

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This study examined the effect of chronic atrazine exposure on anti-predator behavior in freshwater snails (Physa spp.). Fifty adult Physa were collected from Teal Ridge Wetland in Stillwater, OK. Experimental snails (24-hours old) were obtained from the subsequent generation (F1). Fifteen snails were haphazardly assigned to each experimental unit—900mL pyrex bowls. For 21 days, each unit was subjected to one of six treatments: four nominal atrazine concentrations (50, 100, 200, 400 µg/L) or two controls (acetone or water). Following the chronic exposure period, five snails were randomly selected from each treatment replicate (n=4) and separated into individual deli cups for the behavioral assays. To establish baseline responses for each snail, avoidance behavior (above, below, or at the water line) and activity (movement) were scored at 15 minute intervals over a period of 90 minutes. Predator cues—water collected from tanks containing snail-fed crayfish —were then applied to each unit, and the same behavioral metrics were again scored every 15 minutes for an additional 90 minutes. In the presence of such cues, Physa can quickly detect predation risk and crawl out of the water—hence our avoidance metric. Preliminary results suggest that chronic atrazine exposure increases baseline activity, and alters anti-predator behavioral response. These data indicate that exposure to atrazine can alter prey behavior, which has implications for our understanding of the effects of multiple stressors in aquatic systems. Future studies will aim to investigate how atrazine exposure affects epigenetic inheritance of anti-predator phenotypes.

WP307 Photosynthetic capacity of Oedogonium sp. (Chlorophyta) exposed to technical-grade glyphosate and Roundup® treatments

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The macroalgae constitute, usually, dominant autotrophic communities in streams, playing an important role in primary productivity. Although the macroalgae are susceptible to exposure to herbicides coming from agricultural fields near streams and rivers, there are few studies describing the effects of glyphosate on ecophysiological aspects of these producers. Considering this scenario, the present study measured the impact of technical-grade glyphosate and the commercial formulation Roundup® on the photosynthetic response of Oedogonium sp. (Chlorophyta), using data from chlorophyll a fluorescence, oxygen evolution and chlorophyll a content after one (T1) and seven days (T7) of exposure. Three herbicide concentrations were used: i) the maximum concentration for irrigation and animal consumption allowed under Brazilian law (0.28 $mg.L^{-1}$); ii) the recommended concentration to control plagues (3.5 $mg.L^{-1}$); and iii) the highest concentration found in a natural aquatic environment (6.0 $mg.L^{-1}$). Specimens of Oedogonium sp. had improved photosynthetic performance in technical-grade glyphosate treatment at the highest concentrations (3.5 and 6.0 $mg.L^{-1}$) in T1 and T7, and in Roundup® treatment at the lowest concentration (0.28 $mg.L^{-1}$) in T1. However, technical-grade glyphosate treatment showed, for highest concentrations, an increase on non-photochemical quenching in T1, and on quantum yield of non-regulated non-photochemical energy loss in T7, suggesting a damage in the photosynthetic apparatus in long-term. The quantum yield of non-regulated non-photochemical energy loss was also increased in Roundup® treatment for 6.0 $mg.L^{-1}$ in T1, besides the reduction of photosynthetic

efficiency. The results showed that Oedogonium sp. has a relative ability to resist the deleterious effects of technical-grade glyphosate and Roundup® and, depending on the concentration of active ingredient and surfactant, the presence of these herbicides could even enhance its photosynthetic yield. This indicates a probable change in the abundance of this macroalgae due to land use of adjacent areas in which the aquatic ecosystem is located. The comparison between both treatments showed that Roundup® was more harmful than technical-grade glyphosate, mainly in higher concentrations. This result is probably due to the presence of surfactant in Roundup® formulation, which has been repeatedly pointed out as evenly or more toxic than the active ingredient.

WP308 Glyphosate affects finfish cholinesterase activity in acute exposures

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Glyphosate is widely used in Colombia to control illegal crops such as poppy (Papaver somniferum) and coca (Erythroxylum coca) plants as well as weeds of edible crops. Acute (96-h), glyphosate exposures (as Roundup®) in different finfish species of importance in Colombia determined from mild to severe central nervous system (CNS) effects and changes in plasma (nmols/ml/min) and brain (nmols/min/mg protein) cholinesterases (AChE, BChE). Ghost fish specimens (Apteronotus albifrons), an electric fish found in the Orinoco and Amazon rivers basins, exposed to 0, 10 and 90 ppm Roundup® (v/v) (n=27) displayed a significantly higher plasma AChE activity (anova, $p< 0.05$) at 90 ppm Roundup® (112.8 ± 46) as compared to controls (65.0 ± 25.6) and 10 ppm (67.4 ± 26.4). These changes were accompanied by mild CNS signs. Juveniles of red tilapia (Oreochromis sp.) (n=36) exposed to 0, 1, 5, 15, 45 and 90 ppm Roundup® (v/v) showed significant increases in both plasma AChE and BChE at the two highest concentrations (anova, $p< 0.05$) along with severe CNS symptoms in comparison to controls or low-concentration exposures; whereas in Nile tilapia (Oreochromis niloticus) juveniles (n=12), exposed to 0 and 15 ppm Roundup®, there was a significantly lower AChE plasma activity in glyphosate-exposed fish (171.7 ±34.2) as compared to controls (334.5 ± 56.8) (T-test, $p< 0.05$) and no signs in CNS. Interestingly, after 10 days of ceasing the exposure, AChE activity returned to normal baseline levels in those Nile tilapias that had been exposed to Roundup®. Bocachico (Prochilodus magdalenae) (n=12) and yamú (Brycon amazonicus) (n=18), two indigenous fish species of Colombia, showed reduced brain AChE (nmols/min/mg protein) when exposed to 10 ppm (bocachico 1.8 ± 0.6, yamú 7.2 ± 1.8) as compared to controls (bocachico 47.0 ± 4.5, yamú 160.2 ± 13.7) whereas bocachico increased significantly AChE when exposed to 30 ppm Roundup® (113.0 ± 8.0) in comparison to controls and 10 ppm. All the species tested showed changes in cholinesterase activity when exposed to the herbicide. A mechanistic approach to explain interactions between the herbicide and the cholinesterases enzymes remained unknown in our investigations as well as likely ecological implications on fish behavior or interrelations amongst fishes in natural bodies of water due to the presence of glyphosate as a contaminant and the effects on cholinesterase activity.

WP309 Glyphosate (FAENAMR) exposure affects demographic parameters and the size of the progeny in the cladoceran Simocephalus mixtus

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Glyphosate, in different commercial formulations, is the herbicide more frequently used in agriculture to control weeds. As occurs with other pesticides, it can be easily spread by air dispersion or as runoffs, so they can reach aquatic environments and affect non-target species, producing undesirable, toxic effects in the aquatic biota. The objective of this study was to evaluate the effects of exposure to glyphosate (FAENA^{MR}), in the demographic parameters of the cladoceran Simocephalus mixtus, an additionally determine the possible effects in the body size of the progeny released by adults exposed to glyphosate. The EC_{50} was determined; after this, the chronic effect of sublethal concentrations on the demography was determined during 21 days through a Life Table approach; the size of the neonates in consecutive clutches was also determined. The obtained EC_{50} was 5.27 mg L^{-1}. The life expectancy at birth, survival and fecundity were significantly affected in the highest tested concentrations of glyphosate (2.71 and 2.19 mg L^{-1}) with respect to the control. The net reproductive rate and the intrinsic growth rate decreased as the concentration of the herbicide increases, also the clutch size was reduced approximately in 40% in the maximum concentration (4.06 mg L^{-1}). The obtained results show that S. mixtus has great sensibility to the commercial glyphosate formulation, compared with other cladocerans, and warn about the possible toxic effects in non-target species in the aquatic environment.

WP310 The aquatic fate of neonicotinoids under natural irradiation

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Exposure of non-target aquatic species to neonicotinoids has become an increasing concern given the high solubility of this pesticide class. Photodegradation is considered to be the major route of dissipation in aquatic ecosystems; however, little is known about the impact of water constituents on photodegradation in these systems. This study assesses the direct and indirect photolysis of three common neonicotinoid pesticides, clothianidin, imidacloprid, and thiamethoxam. The impacts of several aquatic constituents and pH are being assessed independently and in a factorial design with direct photolysis controls to determine how factor interaction affects photodegradation under natural sunlight. Radical producing and scavenging aquatic constituents found in agroecosystems were chosen including: dissolved organic matter (DOM), nitrate, and iron. A chemical actinometer, p-nitroanisole/pyridine (PNA/PYR), is used in the determination of light intensity and direct photolysis quantum yields. Kinetic models are used to determine the direct and indirect photolytic rate constants and half-lives of the neonicotinoid pesticides. This ongoing study will contribute to the understanding of the aquatic fate of neonicotinoids.

WP311 Evaluation of the toxic effects pesticides from surface agricultural runoff, to the neotropical Cladocera Macrothrix flabelligera

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Brazil is one of the greatest users of pesticides in agriculture, resulting on severe environmental and human health risks. An example of this scenario is found in Bom Repouso/Minas Gerais State, where the insecticide Kraft®(abamectina) and the fungicide Score®(difenoconazol) are applied. Considering the environmental risks, the aim of this work was to evaluate the toxicity of the runoff water contaminated by the pesticides Kraft® e Score®, isolated and in mixture, to the Neotropical cladoceran Macrothrix flabelligera. Aiming to simulate natural conditions of aquatic ecosystems that receiving the input of allochtonous materials from agricultural runoff containing pesticides, the compounds were applied isolated or as a mixture, in different ploughed soil parcels without vegetation by simulating a rain intensity of 19 mm intensity, normally occurring in the rainy period. Doses applied were those recommended by the pesticide industry to strawberry cultures (Kraft®:30 mL/100L of water or 0.1 L/m^2 of solution;

Score®: 0.40 mL/100L, or 0.020L/m^2 of solution). The runoff with all carried materials from uncontaminated control soil and contaminated soil parcels from the various treatments, runoff without the pesticides(R_C), Kraft runoff(R_K), Score runoff(R_S), Kraft+Score runoff(R_{K+S}) was collected and tested in acute toxicity tests (48h) carried out in laboratory for the following dilutions:100%, 75%, 50%, 25%, 12.5%, 6.25%, 3.12%, plus the control using only reconstituted water. Test procedures followed the ABNT NBR13373 protocol for Ceriodaphnia spp. Test organisms. After the exposure period imobile organisms were counted. Results were analyzed by ANOVA followed by Dunnet test. Differences were considered significant when p≤0,05. Values obtained for physical and chemical variables did not differ among treatments, except for turbidity whose minimum and maximum values corresponded to 183 and 380 NTU, respectively. In relation to pesticides toxicity, no significant difference was found between soil runoff without the pesticides(R_C) and soil runoff from Score contaminated parcel(R_S). It was possible to obtainthe IC40 value for Kraft+Score(R_{K+S}) and to establish the IC50 for the 100% Kraft runoff solution. It was demonstrated that agricultural activities promoted the transport of soil particles to the aquatic ecosystems (physical alteration) and that when associated to the use of pesticides (chemical alteration) it can cause deleterious effect to the aquatic biota.

WP312 Ecosystems models (mesocosm) to evaluate the toxicity of the pesticides Kraft and Score in Ceriodaphnia silvestrii (Cladocera)

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Bom Repouso (MG) is a municipality with agricultural characteristics and the production of potatoes and strawberry is relevant to the local economy. Nevertheless, in these crops farmers are using the acaricide/insecticide Kraft® (abamectin) and the herbicide Score® (difenoconazole), isolate or in mixture, and the ecological effects are not being evaluated yet. Considering that, the objective of thsi research was to assess the effects of these pesticides on the Crustacea Ceriodaphnia silvestrii, using studies in aquatic ecosystem models. The experiments were conducted in mesocosms (400 L) containing water, sediment and biological community, simulating the environmental conditions. The pesticides (Kraft and Score) were applied in soil without vegetation, according to the dose recommended by the manufacturer for strawberry crops which was simulated a rain of 19mm. The runoff of water containing all material transported (control and contaminated areas) was collected and placed in mesocosms, simulating a natural condition of contamination, and then water samples were collected for the acute toxicity tests (48h; n=5), under laboratory conditions, according to the NBR 13373 standards for the species C. silvestrii. The results were analyzed by analysis of variance (ANOVA) followed by Dunnett's test at 5% significance level. %. The values of physical and chemical variables were similar in all treatments. However, significant differences were observed for turbidity, in this case the minimum and maximum values were 183 and 380 NTU, respectively, indicating that there is a physical effect, which reduces to 53% and 87% after 24 and 72 hours of application runoff. Regarding toxicity, no significant difference were observed between runoff control, contamination isolated and mixture. This effect can be explained by the short half-life (up to 24 hours in water) of this pesticide, as well as by the adsorption thereof to the particulate material, which promotes its non-availability, reducing the toxic effects on the biological community as mentioned by Novelli et al. (2012).

WP313 Effects of temperature on the toxicity of herbicide sulfentrazone (Boral®SC) in two species of tadpoles in Brazil

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Global climate changes have caused extensive impacts on many species and ecosystems. Changes in environmental temperature can alter the chemical toxicokinects and one current challenge is to predict the risks established by the interactions between non-chemical and chemical stressors at the biological level. Tadpoles can experience large temperature fluctuations in their habitats and many species are distributed in areas impacted by agriculture. Sulfentrazone (Boral®SC) is a widely used herbicide in sugar cane and soy crops in southeastern Brazil. We evaluated the influence of temperature on the toxicity of sulfentrazone in two endemic species of tadpoles, Eupemphix nattereri and Rhinella schenideri, by analysis of oxidative stress biomarkers. Tadpoles were exposed to 0.01, 0.05 and 0.1mg/L at temperatures of 28ºC, 32ºC, and 36ºC during 3 and 8 days. Exposure of tadpoles to sulfentrazone caused changes in antioxidant enzyme activity with temperature-associated responses. At 28ºC, effects of sulfentrazone were only evident in tadpoles of E. nattereri, with an increase in catalase (CAT) and glutathione-S transferase (GST) activity after 3 days of exposure. Glucose-6-phosphate dehydrogenase (G6PDH) was also raised after 8 days in tadpoles of E. nattereri exposed to 0.05mg/L at 28ºC. For both species, the effects of sulfentrazone were more pronounced at higher temperatures. After 3 days of exposure, CAT activity was decreased and G6PDH increased in tadpoles of R. schneideri treated at 32ºC and 36ºC. E. nattereri tadpoles had GST and G6PDH increased when exposed to sulfentrazone at 32ºC and 36ºC. After 8 days, an increase in CAT and G6PDH enzymes was observed in tadpoles of R. schneideri treated at 36ºC. Levels of G6PDH and GST were raised by all concentrations of sulfentrazone tested in E. nattereri tadpoles at 32ºC and 36ºC. Levels of malondialdehyde (MDA) were also induced by the treatments, indicating that the exposure to herbicide sulfentrazone may trigger lipid peroxidation in tadpoles depending on the thermal gradient. Our results showed that temperature is an important variable influencing the toxicity of sulfentrazone in tadpoles and the changes in oxidative stress biomarkers are likely a result of physiological adaptation to stress conditions established by an interaction between chemicals and temperature changes. It suggests that environmental temperature should be considered in risk assessments of environmental contaminants for amphibian species.

WP314 Effects of Diuron and its main biodegradation products on antioxidant and biotransformation enzymes of Nile tilapia

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Diuron is one of the most commonly used herbicide in agriculture, like sugarcane, in São Paulo State. The risk of diuron and its metabolites can represent for aquatic organisms that inhabit rivers and lagoons are not well understood, even though it have been found in many rivers and subterranean water. Diuron, in its original form, have low persistence in the environment, being mainly biodegraded in three other products, dichloroaniline (DCA), dichlorophenyl-urea (DCPU) and dichlorophenyl-methyl-urea (DCPMU), that could also represent a risk for the exposed biota. Considering the extensive use of Diuron in sugarcane crops in São Paulo State and the potential risk of these compounds and its biodegradation products to fish, in this work we measured some biochemical parameters in liver and gills of Nile tilapias, to evaluate the effects that these compound might exert in fish, at environmentally relevant concentrations. The experiment was done by exposing Nile tilapia to 40 and 200 ng/L of Diuron, DCPMU, DCPU and DCA, alone or mixed, for seven days. The activities of catalase (CAT), superoxido dismutase (SOD), glutathione peroxidase (GPx), glutathione reductase (GR), glutathione S-transferase (GST), ethoxyresorufin-O-deethylase (EROD) , and multixenobiotic resistance (MXR) were measured. Our results showed that

diuron and its metabolites, as well as the mixture of all compounds, are prone to alter antioxidant defense system, biotransformation enzymes and first line response protein MXR. We observed an increase in the efflux of the compound out of the cell in groups exposed to Diuron 200 ng/L, DCA 40 and 200 ng/L and the mixture of compounds at 40 ng/L. Furthermore, we observed significant alterations in all enzyme activities assessed, in both tissues. In gill we observed decrease in enzimatic activit and these differences agree with MXR data, except SOD, these correlation is more expressive in GST. According to other authors, the compound efflux can be correlated with decreases in GST activity, as observed in our work. Taken together, our data clearly demonstrated that diuron or its metabolites can impair the Nile tilapia metabolism.

WP315 Influence of temperature on the thyroidogenic effects of Diuron and 3,4-DCA in tadpoles of the American bullfrog Lithobates catesbeianus

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Temperature is a key variable affecting the timing of amphibian metamorphosis from tadpoles to tetrapods, through the production and subsequent function of thyroid hormones. Thyroid function can be impaired by environmental contaminants as well as temperature. Tadpoles can experience large temperature fluctuations in their habitats and many species are distributed in areas impacted by agriculture practice. Diuron is a widely used herbicide frequently detected in freshwater ecosystems throughout the world, and may impact endocrine function in aquatic organisms. We evaluated the influence of temperature on the action of diuron and its metabolite 3,4- dichloroaniline (DCA) on thyroid function and metamorphosis in tadpoles of Lithobates catesbeianus. Tadpoles were exposed to 40ng/L and 200ng/L of diuron or 3,4-DCA at temperatures of 28ºC and 34ºC for 7 days. Both compounds accelerated the metamorphosis of tadpoles maintained at higher temperature. Plasma T_3 concentration was also increased (3-fold) in tadpoles exposed to 200ng/L of diuron at 34°C. Transcripts of thyroid hormone induced bZip protein (thibz) were altered by exposure to both compounds at 28°C. Animals exposed to 200ng/L of diuron and 3,4-DCA at 34°C had higher levels of thibz, deiodinase (dio2 and dio3) and thyroid hormone receptors (trα and trβ) mRNA than animals only treated with contaminants, suggesting an important effect of temperature stress on the expression of these genes. A positive correlation between trβ and klf9 mRNA was observed for both compounds at 34°C. Our results showed that temperature can influence the toxic effects of diuron and 3,4-DCA on metamorphosis pathways of tadpoles by potentially changing concentrations of T_3 and expression of TH-target genes. Whereas changes in global abiotic variables such as frequency of heat waves are estimated to increase in coming years, it is suggested that environmental temperature should be considered in risk assessments of environmental contaminants for amphibian species.

WP316 DNA damage produced by Vinclozolin on two freshwater invertebrates

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The dicarboxiamide Vinclozolin (Vz) is a widely used fungicide for agricultural purposes. Because of this, it is found as a residue in the environment, detected in underground water, lakes, rivers, and seawater. Most of the toxicity studies have been focused on vertebrates showing clastogenic activity, however, few studies have investigated their effects on invertebrates, which are key organisms in aquatic ecosystem maintaining. In this work we have analyzed the effects caused by Vz on two freshwater invertebrates. Fourth instar larvae of Chironomus riparius, a dipteran used frequently as reference species in ecotoxicology, and adults of Physa acuta, a gastropod widely extended, were exposed to this compound for 24 hours to different concentrations of Vz (20 and 200 µg/L). The level of DNA damage was evaluated by measuring different parameters following the comet assay. The results show a significant increase in the different parameters analyzed (%DNA in tail, tail length, tail moment and Olive tail moment) indicating that the presence of Vz affects the integrity of the DNA in these organisms. These results confirm the genotoxicity of this compound on invertebrates and support previous results obtained with micronuclei analysis in P. acuta. Furthermore, this is the first report of the DNA damage on insects by this compound, supporting comet assay as a potential biomarker for genotoxicity in aquatic invertebrates. This work was supported by the Plan Nacional de Investigación Científica, Desarrollo e Innovación Tecnológica (Spain), grant CTM-2015-64913-R from the Ciencias y Tecnologías Medioambientales program. M.A. is the receiver of a predoctoral contract Ministry of Economy and Finance (BES-2013-064041).

WP317 Effects of the fungicide imazalil on the fathead minnow (Pimephales promelas) reproductive axis – a case study in 21st century toxicity testing

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Since its introduction in 1983 imazalil has been used primarily as a fungicide on crops post-harvest, such as tubers and citrus fruits. Its effectiveness lies in the ability to inhibit the fungal enzyme, lanosterol 14 α-demethylase. However, like other azole fungicides, imazalil can inhibit a range of cytochrome p450 enzymes, including one or more involved in steroid biosynthesis. Previous in vitro and high throughput screening assays showed that imazalil can cause aromatase inhibition and reduce 17β-estradiol (E2) production by H295R cells. In the present study, we tested imazalil in a number of in vitro and in vivo bioassays with fathead minnows (Pimephales promelas) to evaluate whether it would elicit effects consistent with an adverse outcome pathway (AOP) linking inhibition of aromatase to reduced fecundity. Ex vivo ovarian E2 and testosterone (T) production by ovary tissue from reproductively mature female P. promelas exposed to imazalil for 24 h at concentrations of 100, 500 and 1580 µg/L were significantly lower ($p< 0.05$) than controls. Plasma E2 concentrations of females exposed for 24 h were significantly lower at imazalil concentrations of 80 and 250 µg/L, but not 2.5, 8, and 25 µg/L. In a separate 60 h exposure, plasma E2 concentrations were significantly lower than controls in mature P. promelas exposed to 200 µg imazalil/L, but not at 0.2, 2, or 20 ug/L. Real-time quantitative PCR analyses measuring relative abundance of mRNA transcripts for various CYPs in ovary indicated an upregulation of cyp19a1a, cyp17, and cyp11a in P. promelas exposed to 200 µg imazalil/L. This induction is potentially due to compensatory/feedback responses along the hypothalamic-pituitary-gonadal axis. Overall, data collected to date support the hypothesis that imazalil causes endocrine disruption in exposed female fathead minnows in a manner consistent with an established AOP and supports the broader utility of that AOP in hazard assessment. The contents of this abstract neither constitute nor necessarily reflect USEPA policy.

WP318 Toxicity of three anticoagulant rodenticides in non-target fish species

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Bird colonies at certain US Fish and Wildlife Service (USFWS) Pacific Island National Wildlife Refuges (PINWR) have suffered severe population losses due to rat infestations. Anticoagulant rodenticides (ARs) are a common tool used for rat eradication and control programs to protect native species at PINWR. In one such aerial broadcast of AR bait on Lehua Island, Hawaii, a 'fish kill' was reported on a neighboring island, Niihau Island. Reports were anecdotal, but investigations by the state conservation agency, Department of Land and Natural Resources (DLNR), and Division of Aquatic Resources determined a few species of fish were dead and potentially more were exposed. Due to lack of AR toxicity data in fish; it is difficult, if not impossible, to assess risk to fish. Therefore, our objective was to determine sensitivity of three AR chemicals: diphacinone, chlorophacinone, and brodifacoum in triggerfish, specifically, red-toothed triggerfish (Odonus niger) and black triggerfish (Melichthys niger). Due to low sample size, the Up and Down Procedure (UDP) was used to determine the median lethal dose. Preliminary red-toothed triggerfish LD50 results: brodifacoum (36-48 µg/g body weight, n=4) diphacinone (137-175 µg/g, n=4) and chlorophacinone (132-182 µg/g, n=3). Across-taxa Species Sensitivity Distribution (SSD) was used to model the variation of species' sensitivity to AR chemicals. The red-toothed triggerfish were among the least sensitive species to AR chemicals as compared to other taxa, and in particular, mammals and birds.

WP319 The effects of quercetin, a potential natural product pesticide, on zebrafish development

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Quercetin, a member of the flavonoid family, is a natural product that has also been shown to have synergistic effects when combined with insecticides such as organophosphates and pyrethroids. In addition to its potential use as an insecticide, quercetin has been used in food applications because of its potential anti-oxidant and other beneficial health properties. However, quercetin has also been shown to impair synaptic transmission and may have carcinogenic properties, thus data are controversial as to its benefits as a pesticide or a health supplement. To learn more about the toxicity of quercetin to non-target organisms, we exposed zebrafish embryos to three concentrations of quercetin (1, 10 ,100 µg/L) for 96 hours. Quercetin did not appear to induce significant mortality in zebrafish over 96 hours, but there was an increased prevalence of dorsal curvature in larvae in a concentration-dependent manner. No other gross deformities, such as pericardial edema nor yolk sac edema, were observed. As mitochondrial function is important for development, we aim to further explore the effects of Quercetin exposure on mitochondrial respiration using the Seahorse XFe24 Extracellular Flux Analyzer, to understand the interaction between ATP production and development which may be modulated by Quercetin. This study aims to determine whether there are significant developmental defects in fishes due to Quercetin, to provide a foundation for ecological risk assessment.

WP320 The evolution of tolerance to pesticides: insights from resurrection ecology

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Previous research has demonstrated that resurrected genotypes from an ancestral population of Daphnia pulicaria have differential toxicity (median effect concentration – EC50) to the insecticide chlorpyrifos. Ancient genotypes (1301-1646 A.D.) were on average more sensitive to this chemical when compared to the contemporary genotypes (1967-1977, 2002-2008 A.D.). In order to determine the physiological mechanisms of tolerance, a series of biochemical assays was performed on the three most tolerant and three most sensitive genotypes from the population. In vitro acetylcholinesterase assays yielded no significant differences in constitutive activity or oxon-binding sensitivity. Acute toxicity tests were conducted using i) the toxic metabolite chlorpyrifos-oxon (CPY-O) and ii) CPY-O co-applied with piperonyl butoxide (PBO), a known Phase-I metabolic inhibitor. Both series of toxicity tests reduced the mean variation in sensitivity between tolerant and sensitive genotypes. Exposure to CPY-O reduced the disparity from a 4.7-fold to 1.6-fold difference in sensitivity. The addition of PBO further reduced the variation to a 1.2-fold difference in sensitivity. This suggests that pathways involving Phase-I detoxification and/or bioactivation of chlorpyrifos play a significant role in dictating tolerance in this population. Future work on this system will aim to illuminate the functional basis of metabolic tolerance via next generation RNA sequencing.

WP321 Effects of S-metolachlor and its typically paired safening agent (benoxacor) in C. riparius: 28-day chronic exposure study

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The environmental effects of safeners, chemicals that protect crops from herbicide toxicity, are largely unknown. Safeners are considered inert ingredients added to many major classes of herbicides. Under controlled anaerobic conditions in the laboratory, dichloroacetamide safeners can degrade into potentially toxic byproducts. This study's purpose was to determine the toxicity of the dichloroacetamide safener benoxacor, a degradation product of benoxacor (mono-chlorinated benoxacor), a typically paired active ingredient, S-metolachlor, and a simulated formulation (a mixture of S-metolachlor and benoxacor) to the aquatic, sediment-burrowing larvae of Chironomus riparius. Larval C. riparius were exposed to these four chemicals in chronic 28-day experiments within environmentally relevant microcosms. The results demonstrated that high concentrations of all four chemicals affected percent adult emergence (survival), and time-to-emergence (also known as time-to-event) curves. High concentrations of benoxacor and the mixture affected adult emergence rates, and high concentrations of S-metolachlor affected male adult dry body mass. The present study's results demonstrate that benoxacor and monochlorinated benoxacor are toxic to C. riparius at similar concentrations as the herbicide S-metolachlor, which is useful information for the better understanding of the ecotoxicological effects of dichloroacetamide safeners. The movement and equilibration concentrations of benoxacor were also demonstrated under replicated microcosm conditions in a separate experiment over 28 days.

WP322 Organic and inorganic contaminants in white shrimp from the St. Johns River, Florida

J. Higman, John Higman

Concentrations of organic and inorganic (metal) contaminants were measured in immature adult white shrimp collected by otter-trawls from four locations in the St. Johns River, Florida. Thirty to forty of the larger shrimp were selected from each location. Selected individual shrimp were decapitated and cephalothoraxes ("heads") were stored separately from abdomens ("tails") in pre-cleaned glass jars with Teflon lids. Penaeus shrimps are "quasi-catadromous" living much of their lives in estuaries. After hatching in the ocean, small postlarval Penaeus shrimp move into shallow estuarine nursery areas in bays, lagoons, rivers, tidal creeks, salt marshes. Postlarval shrimp continue to mature in the estuaries, molting and growing through juvenile stages in 2 to 6 months. As immature adults they begin migrating to the ocean where they mature. Therefore, immature adult shrimp caught before migration would have accumulated contaminant burdens in the estuaries where they were captured. Results for the 31 pesticides, 27 individual PAHs (polynuclear aromatic hydrocarbons), 23 individual PCB (polychlorinated biphenyl) congeners, 8 chlorinated industrial compounds and 11 metal contaminants were evaluated and discussed if concentrations were above analytical detection levels. The whole body contaminant concentrations calculated by summing cephalothorax and abdomen data from each site are also presented: (e.g. total mercury ranged from 11.9-13.8 µgHg/kg wet wt. (51.9-61.5 µgHg/kg dry wt.); (e.g. sum of 7 chlordanes ranged from 1.88-7.94 µgCdane/kg wet wt. (8.08-38.23µgCu/kg dry wt.) Shrimp samples at all four sites had low level measurable concentrations of PCBs, PAHs, derivatives of chlordane, DDT and traces of chlorinated benzenes in cephalothorax composites. Most of these compounds were also found in abdomen composites, but only in trace quantities. Chromium was the only metal not found in any of the abdomen or cephalothorax composite samples. In general, the majority of the shrimp contaminant burdens were associated with the cephalothorax, and typically less than 50% of the whole body value was detected in the abdomens.

WP323 Effects of species characteristics on bioaccumulation of polychlorinated biphenyls (PCB) in a boreal freshwater lake

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Equilibrium partitioning of hydrophobic organic chemicals (HOCs) drives chemicals towards equilibrium between the biota and their environment. However, several biological characteristics modify bioaccumulation resulting species specific body residues of HOCs. In addition, within species, age-, sex- or habitat-specific changes in body residue may take place e.g. due to changes in diet, capability for metabolation, or changes in concentrations of HOCs in the environment. This study explored the polychlorinated biphenyl (PCB) contaminated freshwater lake, and studied the species-specific and within-species differences in bioaccumulation in different taxa from invertebrates to top predators. The results generally indicated species-specific differences in correlation of PCB profile between the tissues and the source phases. The PCB profile of free-swimming macroinvertebrates correlated best with the profile of the water phase indicating bioconcentration as the main phenomena of the bioaccumulation. On the other hand, the PCB-profile of the higher predator fish species correlated best with the profile of their diet content, indicating the diet being the main source of bioaccumulation for HOCs. Closer examination of perch and roach revealed that metabolation capability changes during the life span, and that source of nutrition determined the biomagnification of HOCs being different between the species. As a conclusion, the biological characteristics of species modify strongly bioaccumulation of HOCs. Thus species composition in the given ecosystem, and hence available prey selection composes a unique bioaccumulation scenario for each food web.

WP324 Histopathology of Brown Bullhead, Smallmouth Bass, and Yellow Perch in Relation to Polychlorinated Biphenyl (PCB) Contamination in the Hudson River

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As part of the Hudson River Natural Resource Damage Assessment, the Trustees conducted a Fish Health Assessment study that evaluated the prevalence of toxicopathic lesions in adult brown bullhead (Ameiurus nebulosus), smallmouth bass (Micropterus dolomieu), and yellow perch (Perca flavescens). Fish were collected in September and October 2001 from each of four locations: two highly contaminated areas below the plants (Thompson Island Pool (TIP) and Stillwater Dam Pool (STW)), an upriver reference area (Feeder Dam Pool (FDP)), and a reference lake, Oneida Lake (ODA). The focus was on histopathologic lesions and observations associated with contaminant exposure: liver—neoplasms, foci of cellular alteration, bile duct hyperplasia; testes—ovotestis (testicular oocytes), germ cell degeneration, altered developmental stage; ovaries—atresia and altered developmental stage. Lesions associated with PCB exposure were defined as those with significantly greater prevalence and/or severity in TIP and STW collections compared with those from ODA and FDP. Results will be discussed.

WP325 Assessing potential endocrine disruption in largemouth bass from a PCB-contaminated reservoir in South Carolina

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Endocrine disruption is the abnormal modulation of normal hormonal physiology by exogenous compounds. In fishes, endocrine disruption of the reproductive system has been documented frequently throughout the Southeastern United States and worldwide. Many studies of endocrine disruption in fishes have looked at the effects of wastewater effluent or pulp and paper mill effluent as these are known sources of endocrine disrupting chemicals (EDCs). Another EDC that has not definitively been linked to endocrine disruption in fishes are polychlorinated biphenyls (PCBs). In this study, we examined if PCBs influence endocrine disruption, vitellogenin production, and intersex condition in wild largemouth bass, Micropterus salmonides (LMB). Portions of Lake Hartwell in South Carolina have been heavily contaminated with PCBs since the 1970's warranting additional research on biological responses to PCB contamination. LMB were collected from two sites on Lake Hartwell and a reference site upstream at Lake Keowee. Vitellogenin (Vtg), the egg-yolk precursor protein, and intersex condition in the form of testicular oocytes (TO) were measured and evaluated in LMB. In LMB fillet samples, average PCB concentrations at the impacted site on Lake Hartwell remain above 2 ppm. Elevated Vtg levels above the measurable detection limit (0.001 mg/ml) were found in male fishes collected from both Lake Hartwell and Lake Keowee. Intersex was also found in male fishes collected from both lakes. This is the first study to our knowledge that specifically looks at endocrine disruption in wild LMB from Lake Hartwell and Lake Keowee in South Carolina and will add to the understanding of the extent of endocrine disruption, possible EDC sources, and legacy effects of PCBs on fishes.

WP326 Evaluating the Role of UV Exposure and Recovery in PAH Photo-Induced Toxicity

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Photo-induced toxicity refers to the ability of a molecule to increase in toxicity in the presence of solar radiation. Polyaromatic hydrocarbons (PAHs) are one class of compounds known to be phototoxic. Several models for PAH phototoxicity have been developed that predict toxicity as a reciprocal product of PAH concentration, UV intensity, and exposure time. However, these models do not incorporate biological recovery time as a factor. Aquatic organisms are exposed to UV light in daily cycles where light exposure is inconsistent due to various environmental factors. Thus, inclusion of non-UV recovery time periods as factors in predictive models will increase their ecological relevance. The first objective of this research was to evaluate toxicity when UV is distributed in even intervals. Daphnia magna neonates were exposed to fluoranthene in four different concentrations and UV exposure intervals between assays included 24 hrs, 12 hrs, 6 hrs, 3 hrs, and 1.5 hrs. The second objective of this research was to evaluate toxicity when UV is distributed in uneven intervals. D. magna neonates were exposed to the same four fluoranthene concentration as in the first objective and UV exposure intervals varied between assays with inconsistent intervals of 12 and 6 hrs, 9 and 3 hrs, 9 and 1.5 hrs, 6 and 3 hrs, 3 and 1.5 hours. For both objectives, phototoxic dose remained consistent across all assays and mortality was assessed at 24 and 48 hrs. Here we show that LC50 values differ between even and uneven UV interval tests and that increasing recovery interval frequency significantly reduces mortality. In assays where recovery time intervals were in short frequent intervals LC50 values increased up to 3 times than assays where recovery were in long infrequent intervals. This research demonstrates how UV recovery time is an important consideration for photo induced toxicity model development.

WP327 The photo-induced toxicity of polycyclic aromatic hydrocarbons and a lubricating oil in two amphibian species, Xenopus laevis and Rana sylvatica

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Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous environmental contaminants derived primarily from the incomplete combustion of organic matter. PAHs are generally not acutely toxic to aquatic organisms; however, ecologically relevant intensities of UV light have been shown to increase the acute toxicity of certain PAHs. Early life-stages of amphibians may be particularly susceptible to PAH photo-induced toxicity as they are translucent, have permeable skin and undergo embryo and larval development in shallow ponds. Limited studies have investigated the potential photo-induced toxicity of PAHs in amphibian species making it difficult to predict the impact of individual PAHs and complex mixtures containing PAHs. The objective of the present study was to evaluate and compare the sensitivity of a model organism (Xenopus laevis) and an ecologically native species (wood frog, Lithobates sylvaticus) to the photo-enhanced toxicity of PAHs as well as a petroleum-based lubricating oil, UNIVIS J13, known to contain a mixture of PAHs. 96-h tests were performed in which tadpoles were exposed to individual PAHs (anthracene, naphthalene, acridine, benzo(a)pyrene) or UNIVIS J13 for 8 hours in the dark, transferred to clean water, and then exposed to UVA (315-400nm) / UVB (285-315nm) light for 12 hours. Results were compared using mortality, growth, body burden, and whole body transcriptomic responses. In the presence of UV light, 100 ug L^{-1} benzo(a) pyrene significantly increased tadpole mortality, and 200 ug L^{-1} anthracene significantly increased tadpole mortality and decreased tadpole length.Acridine and naphthalene were not found to significantly affect tadpole mortality or growth at any concentrations tested. The co-exposure of UV light and 1:1, 1:10 and 1:100 loadings of UNIVIS J13 water accommodated fractions (WAF) had no significant effect on tadpole mortality or growth. Based on mortality data, it appears that Xenopus is more sensitive to the photo-induced effects of PAHs compared to the wood frog. This may establish Xenopus as a protective model for native species of amphibians with regards to the photo-induced toxicity of PAHs. Overall, anthracene and benzo(a)pyrene exhibit photo-induced toxicity in larval amphibians while such toxicity is not demonstrated by UNIVIS J13, a petroleum-based mixture containing PAHs.

WP328 PAHs and Ultra Violet light DNA damage in Artemia franciscana

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Polycyclic aromatic hydrocarbons are a group of substances with a wide environmental distribution; they can cause oxidative stress and DNA damage. The risk of these effects increases when exposure is combined with solar UV radiation. The objective of the present work was to evaluate DNA damage in Artemia franciscana exposed to fluoranthene and benzo(α)pyrene. Metanauplii of A. franciscana were used in a sublethal test where DNA damage was evaluated with the single gel electrophoresis method (comet assay). Two concentrations of fluoranthene (24 and 48 µg/L) and benzo(α)pyrene (4.8 and 9.6 µg/L) were tested, with 8 replicates of 100 organisms each. Two controls were included: one with sea water only, and another with acetone. A light control t was also run dfor each PAH Exposures lasted 24 h with a 12:12 photoperiod. Results indicated that the largest comets tails and the highest percent damage were obtained in organisms exposed to benzo(α)pyrene. in both concentrations. Fluoranthene, benzo (α)pyrene and anthracene are genotoxic for A. franciscana in the concentrations used for this work, the comet assay was a sensitive method and confirm that exposure to hydrocarbons and UVR damage the DNA chain and can be lethal for nauplii.

WP329 PAH-induced changes to gene expression in the gill and liver of the Atlantic stingray, Dasyatis sabina

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Many elasmobranchs are keystone species that maintain balance within their environments, therefore conservation of these taxa is important to ecosystem health. Additionally, numerous elasmobranch species reside in the northern Gulf of Mexico, including 22 species listed as critically endangered, endangered, or vulnerable by the International Union for the Conservation of Nature. The distribution of these organisms is coincident with areas of heavy mineral exploration, however there is limited knowledge regarding the effects of PAH exposure on elasmobranchs. To investigate these effects we performed ex vivo incubations of liver and gill tissues from the Atlantic stingray, Dasyatis sabina. Three D. sabina were captured via trawl in the MS sound and acclimated to the lab for two weeks. Organisms were sacrificed using MS-222, liver and gill tissues harvested, and tissues incubated for 0.5, 6, or 12 hours in media of 0%, 5%, or 50% high energy water accommodated fraction (HEWAF) prepared with Elasmobranch Ringer's solution. To track changes in tissues over time, HEWAF-related alterations in gene expression of energy metabolism and osmoregulatory genes were measured using qPCR, and further gene expression changes were analyzed via RNA seq. These data represent the first report of changes in elasmobranch gene expression related to PAH exposure and further provide information useful for predicting oil spill impacts to elasmobranch populations.

WP330 Effects of dietary benzo[a]pyrene exposure on wood frog (Lithobates sylvaticus) tadpoles

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Recent large-scale amphibian population declines around the world have prompted investigation of the impacts of environmental contaminants on amphibian health and performance. Many toxicity studies focus on the model amphibian species, Xenopus laevis, and little toxicological data available for native North American species. Benzo[a]pyrene (B[a]P) is a polycyclic aromatic hydrocarbon that is widely found in the environment and is known to induce oxidative stress and detoxification pathways in mammals and fish; however, few studies examine the impacts on native North American amphibian species. B[a]P is also known to adsorb onto particles present in the water column, making dietary exposures ecologically relevant. In this study, pre-metamorphic tadpoles (Gosner stage 30-32) were provided food ad libitum that was spiked with 50 µg/g and 200 µg/g B[a]P for 14 days. Mortality was observed daily and following the 14-day exposure individuals were assessed for developmental stage, length, total weight, liver and gut weights, and sampled for histology (whole body and gut), antioxidant defense parameters (total glutathione, glutathione peroxidase, superoxide dismutase) and gene expression analysis of liver detoxification pathways (cytochrome P450 and glutathione s-transferase). There was no significant effect of a 14-day dietary exposure to B[a]P on body weight, organ weights or length of the tadpoles. Histopathology and gene expression analyses are ongoing but are expected to provide insight into the risks and health detriments of B[a]P exposure in a wood frogs through an ecologically relevant route of exposure.

WP331 Developmental sensitivity to benzo[a]pyrene exposure and changes in expression of immune-related genes in the amphibian Xenopus laevis

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The need to understand contaminant-induced immunotoxicity in amphibians is evident given recent disease-driven population declines around the world. The amphibian immune system is dynamic over development; early embryo-larval life stages rely primarily on the innate immune system until the adaptive immune system begins to increase in function during prometamorphosis. Therefore, the impacts of environmental contaminants on the immune system may depend on the stage of development at the time of exposure. As a key component of the innate immune system, cytokines are among the first to respond to viral pathogens by mounting an inflammatory response and analysis of cytokine mRNA levels has been used to predict the immunomodulatory potential of chemicals. The current study assessed the response of pro-inflammatory cytokines (tumor necrosis factor α (TNF-α), interleukin -1β (IL-1β) and interferon-γ (IFN-γ)), cytochrome P450 1A1 (CYP1A1) and aryl hydrocarbon receptor (AhR) to benzo[a] pyrene (B[a]P) exposure at various stages of amphibian development. B[a]P is a model polycyclic aromatic hydrocarbon and a known immunotoxicant in mammals and fish; however, little immunotoxicity data is available for amphibians. Embryos were exposed for 96 hours to sub-lethal concentrations (10, 175 and 350 μg/L). Similar exposures for individuals at stage 51 (pre-metamorphosis) and 56 (pro-metamorphosis) were performed using the same concentrations for 7 days (sub-sampling at day 2). Individuals were examined for morphological abnormalities, lengthed, weighed and sampled for gene expression analysis. Impacts on morphological endpoints (abnormalities, length and weight) were more pronounced in earlier life-stages with a dose-dependent decrease in total length and tail length in embryos and no effects in pro-metamorphic tadpoles. Additionally, pre-metamorphic tadpoles exposed to B[a]P had lower TNF-α expression and no change in IL-1β while the opposite was true for pro-metamorphic tadpoles. This research will help us understand mechanisms underlying developmental stage-specific immunomodulatory effects of B[a]P exposure in amphibians. Reducing the efficiency of innate immune defense mechanisms during the tadpole life-stage could increase the susceptibility to infectious agents and contribute to disease-driven population declines.

WP332 Combined Effects of Polycyclic Aromatic Hydrocarbons and Other Environmental Stressors on Fundulus grandis

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In the spring of 2010, the Deepwater Horizon oil spill released > 3 million barrels of crude oil into the Gulf of Mexico. A major component of crude oil are polycyclic aromatic hydrocarbons (PAHs). Although the toxicity of PAHs to fish has been studied to a large extent, the combined effects of extreme abiotic factors and oil are poorly understood. Fundulus grandis larvae (< 24 hours post hatch) were exposed to varying environmental conditions (dissolved oxygen 6 ppm, 2ppm; temperature 20, 25, 30?C; and salinity 3, 10, 30 ppt) combined with varying concentrations of high energy water accommodated fractions (HEWAF) (total PAHs 0 – 140 ppb) for a total of 48 hr. High temperature and salinity resulted in the lowest survival. Surviving larvae were photographed and videos of the heart at high magnification taken. There were notable abnormalities of the heart supporting previous studies showing PAH effects on the cardiovascular system. Expression of the hepatic detoxifying gene cyp1a was highly induced in PAH-exposed larvae, but only in the high dissolved oxygen conditions. A subset of larvae will be processed for transcriptomics using next generation sequencing based on phenotypic cardiovascular changes and cyp1a expression levels. This data will be used to identify which combination(s) of environmental conditions and PAH levels are most likely to result in long-term physiological effects and therefore negatively affect population recruitment.

WP333 Dietary Exposed to Weathered Iranian Heavy Crude Oil : Effects on the detoxification and immune system in Rockfish (Sebastes schlegeli)

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This study examines whether acute toxic responses played by the immune system depend on which metabolic pathway was induced after exposure to weathered crude oil. We exposed juvenile rockfish to weathered crude oil (Iranian Heavy Crude Oil; WIHCO in gelatin capsules) from the "Hebei spirit" oil spill by feeding. The effects on fish hepatodetoxification enzymes (Cytochrome P4501A) and the expression level of the immune response genes, including interleukin-1beta (IL-1β), nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB), Interferon-stimulated gene 15 (ISG 15) and granulocyte colony-stimulating factor (GCSF), were measured in the liver and kidney. The WIHCO-fed fish had significantly higher concentrations of biliary fluorescent metabolites and CYP1A expression during the initial stage after exposure (6 h after exposure) than those of the control group. Similarly, expression levels of IL-1β, p53, NF-kB and ISG15 mRNA were detected highly in the kidney at the early stages of exposure (6 h after exposure). In this study, the apoptosis, phagocytosis activity and cell cycle arrest analysis of juvenile rockfish exposed to WIHCO were measured by flow cytometry, a novel approach. Our results demonstrated that WIHCO caused an increase of apoptosis and cell cycle arrest and inhibition of phagocytic activity in juvenile Rockfish. Several immune-related genes in oil-fed fish were induced at the initial stage of exposure to the WIHCO but decreased to less than that of the control group after the initial stage of response. Therefore, It can lead to a better understanding of impact on marine fish exposed to lingering oil may allow the pathogens, including the infectious diseases, to more easily affect fish.

WP334 A comparative study of RNA-seq analysis on two marine embryonic fish exposed to Iranian Heavy Crude Oil

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This study clarifies the comparative developmental effects of Iranian heavy crude oil (IHCO) on the different toxic sensitivities of transcriptional responses between fish species and the weathering status of crude oil. We utilized high-throughput (Illumina RNA-seq) to characterize the developmental toxic effects from oil exposure. The assembled contigs contain 66,070 known unigenes in flounder and 76,498 known unigenes in spotted seabass. Comparison of different gene expression profiles reveals that the numbers DEGs are differentially higher up and down-regulated in embryonic seabass than those of embryonic flounder exposed to fresh IHCO (FIHCO) and weathered IHCO (WIHCO). Gene pathway analysis from the most differentially expressed gene set was classified from crude oil exposure: indicated the oxidative phosphorylation, disease pathway (Parkinson's, Alzheimer's, Huntington's disease) and cardiac muscle contraction. The expression patterns of 13 differentially expressed genes were validated by quantitative real-time RT-PCR (average correlation cutoff p< 0.005). Especially, the level of genes involving detoxification (CYP1A, CYP1B1 and CYP1C1) in embryonic seabass increased higher than those of flounder but the other genes involving cell processing, development and immune system changed higher in flounder exposure to FIHCO and WIHCO. In differentially orthologous gene clusters analysis, embryonic flounder seem likely to be affect similarly from FIHCO and WIHCO exposure but embryonic seabass is more likely to be affect from WIHCO exposure. These findings support that the severity of developmental defects was greater in flounder compared to that in sea bass. As found in our previous studies, we suggested that the species-specific differences should consider in risk assessment from resident fish species after oil spill incident. Additionally, our work extends the prior understanding of the role of nkx in embryonic heart morphogenesis in exposed to FIHCO and WIHCO in marine fish species. Over all, this information provide a useful new biomarker resource for future studies of effects on embryonic marine fish, which will facilitate future understanding of toxic molecular mechanisms on developing fish exposed to crude oil.

WP335 Effects of Three Crude Oil Aromatic Constituents on a Model Fish Species Anaplopoma fimbria

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When an oil spill occurs in the marine environment, the oil will either be mechanically collected, burned off, chemically broken down, or naturally be broken down by organisms in the water column. The actual composition of crude oil is a hydrophobic mixture of hydrocarbons consisting mostly of alkanes, cycloalkanes, and various mono and polycyclic aromatic hydrocarbons. Three aromatic hydrocarbon constituents found in many crude oils are phenanthrene, 2-methylnapthalene and toluene. These compounds are representative of tri, di and mono aromatic hydrocarbon classes that pose an aquatic toxicity concern. In this study, we have investigated the acute toxicity of these hydrocarbons to the juvenile deep-sea fish species (Anaplopoma fimbria). For each individual chemical, a range finding test was first conducted to determine the appropriate range of exposure concentrations that reduced survival of these test organisms. Then, testing was performed in closed systems over a 96-h period with four concentration levels, which depending on test compound, ranged from 0.05 mg/L to 15 mg/L. Analytical confirmation was performed to document exposure concentrations in each test. Mortality was recorded every 24 h and the LC50 of each chemical determined. Consistent with previous data, our results show that these aromatic hydrocarbons negatively impact fish survival as a function of the Log Kow of the test compound The 96-h fish LC50 was 0.28, 0.89, 6.84 mg/L for phenanthrene, 2-methyl naphthalene and toluene, respectively.

WP336 The comparative acute toxicity of crude oil, oil industry chemicals and refined petroleum products on Paleomonetes africanus

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A comparative assessment of the acute toxicity of Crude oil, Oil industry chemicals(corrosion inhibitors and drilling mud) as well as Refined Petroleum Products (Diesel and Petrol) acting singly against brackish water shrimp paleomentes afrianus obtained from the Lagos lagoon was investigated. The results of 96hrs acute toxicity indicated that diesel (LC_{50}=0.33mg/l) was the most toxic chemical substance followed by crude oil (LC_{50}=0.504mg/l), petrol (LC_{50}=0.646mg/l) and corrosion inhibitor (LC_{50}=3.978mg/l) while drilling mud (LC_{50}=1029000mg/l) was the least toxic. The computed toxicity factor(TF) indicated that diesel was 1.5,1.9, 11.9 and 30×10^4 times more toxic than crude oil diesel, corrosion inhibitor and drilling mud respectively. The findings from this study imply that crude oil, petroleum products and oil industry chemicals pose threat to sensitive aquatic species. Therefore crude oil exploratory, refining and transportation process should be executed in aquatic ecosystems in line with the best available practices to minimize negative effects on aquatic organisms.

WP337 Potential biomarkers of perfluorinated alkyl acid exposure in dolphin embryonic kidney cells

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Perfluorinated alkyl acids (PFAAs) are anthropogenic contaminants that have been found in high concentrations in marine mammals worldwide. Perfluorooctane sulfonate (PFOS) is typically the dominant PFAA found in wildlife, while the majority of other PFAAs are long-chain perfluorinated carboxylic acids (PFCAs) with 8 carbons or more. However, the mere presence of these chemicals is not sufficient evidence to conclude the animal will experience negative health impacts. Investigating cellular changes in RNA and protein expression after exposure to a xenobiotic can help to deduce the potential mechanism of action and can also lead to the development of biomarkers of exposure and effect that can be used in health risk assessment. A majority of the cellular response to PFAAs has been shown to be mediated by peroxisome proliferator-activated receptors (PPARs), and specifically PPARa has been shown to regulate the transcription of cytochrome P450 4A (CYP4A) enzymes. Additionally, the constitutive androstane receptor (CAR) plays a vital role in the regulation of cytochrome P450 2B (CYP2B) enzymes. Both enzymes are necessary for the metabolism of endogenous lipophilic substances, as well as drugs and other xenobiotics, such as PFAAs. Previous observations in stranded cetaceans showed a significant positive correlation between certain long-chain PFAAs, PPARa mRNA and CYP4A protein expression in the kidney, while a relationship between PFAAs, CAR and CYP2B has yet to be evaluated. The purpose of this study was to evaluate the ability of PFAAs with a carbon chain length of 8 or greater to stimulate a cellular response in dolphin embryonic kidney cells (CDK), along with human embryonic kidney cells (HEK), and to ascertain the possibility of using that response as a measurable biomarker of PFAA exposure in vivo. The cytotoxic effects of 8-11 carbon PFAAs, including PFOS, PFOA, PFNA, PFDA, and PFUnA, were determined by incubating the cells with increasing concentrations of each compound for 4 to 72 hours and measuring cell viability with the Trypan blue dye exclusion assay. To determine the ability of each PFAA to activate PPARa, CYP4A, CAR and CYP2B, the dose-dependent response in mRNA was measured using real-time PCR and protein expression was measured using Western blot. Confirming a relationship between PFAA exposure and a specific cellular response in cetaceans will improve our understanding of the potential health impacts from these emerging contaminants.

WP338 Is PFHxA a good alternative to PFOA, based on a toxicity assessment of morphometric, behavioral, and gene expression endpoints in zebrafish?

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Perfluoroctanoic acid (PFOA) and perfluorohexanoic acid (PFHxA) belong to the class of perfluoroalkylated substances (PFASs) which are extremely stable and resistant to biodegradation. These compounds have been detected in human, wildlife, and environmental samples worldwide. Both compounds consist of a fully fluorinated carbon backbone with a carboxylic acid end group. PFOA contains 8 carbon atoms in its backbone, whereas PFHxA contains a 6 carbon chain. PFOA was produced from 1950 to 2000, when a voluntary phase-out began. PFHxA was introduced as an alternative in manufacturing processes, in part, due to its shorter half-life in mammals in comparison with PFOA. Previous studies from the literature, as well as from our lab, have shown exposure to PFOA and PFHxA in zebrafish to result in both developmental defects and reduced survivorship. In the current study, zebrafish were exposed to PFOA or PFHxA (0, 0.1, 2.0 μM) for five days post fertilization (dpf). Morphometric, behavior, and gene expression end points were assessed at the sac-fry (5 dpf), larval (14 dpf), and adult (90 dpf) stages. At 5 dpf, both PFOA and PFHxA exposure resulted in significant increases in yolk sac size and interoccular distance, and significant decrease in total body length. However, at 14 dpf, PFOA-exposed zebrafish (14 dpf) remained significantly smaller in length, whereas there was no difference in total body length in the PFHxA-exposed larvae compared to the controls. In addition, the PFOA-exposed larvae showed an increase in total distance swam and swimming velocity in behavior assays. Gene expression analysis at all stages (5 dpf, 14 dpf, and 90 dpf) indicated a decrease in expression of organic anion transporting polypeptides (slco2b1, slco1d1, slco3a1, and slco4a1) in PFOA- and PFHxA- exposed fish. The short-term, embryonic exposure to PFOA resulted in negative outcomes in terms of growth, development, and behavior throughout critical life stages. At similar low doses these outcomes were observed in PFHxA-exposed zebrafish as well. The persistence of these effects following a brief 5 day exposure adds to the data raising question to the use of PFHxA as a safer environmental alternative to PFOA.

WP339 The cardiorespiratory and metabolic effects of acute naphthalene and pyrene exposure in adult zebrafish (Danio rerio)

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Polycyclic aromatic hydrocarbons (PAHs) are aquatic contaminants often originating from anthropogenic sources. Naphthalene (NAP) and pyrene (PYR) are important petrogenic PAHs that are not as well studied as the prototypical PAH, benzo-a-pyrene (BaP). We hypothesized that acute exposure (48-hours) to NAP and PYR will cause sublethal cardiorespiratory and metabolic impairment similar to that observed in previous studies after acute BaP exposure in adult zebrafish (Danio rerio), but by different mechanisms. To investigate this hypothesis, adult zebrafish were aqueously exposed to PAHs (NAP, 0, 3.7, 370, and 3700 µg/L; PYR, 0.025, 2.5 and 25 µg/L) using static renewal (24h) and compared to dimethysulfoxide controls. No mortalities were observed in any treatment group. At 48h, fish (n=16 fish/group) were subjected to high frequency cardiac ultrasound. The ratio of the atrial contractile rate to ventricular rate (AV ratio) and stroke volume (SV) increased in both NAP- and PYR-exposed fish. This higher AV ratio is indicative of an atrioventricular conduction block similar to that observed with BaP in previous studies. However, while the atrial contraction rate for PYR was unchanged compared to control, NAP atrial rate was higher, likely representing an increased adrenergic tone on the heart. In addition, after NAP exposure, a large increase in end diastolic volume (EDV) was noted, but with a lower ejection fraction, indicating some impairment in ventricular contractility despite higher preload mediating great cardiac filling. The higher EDV observed with NAP, but not PYR, may be related to gill irritation and whole body edema observed with NAP. The acute effects of NAP and PYR on swimming endurance and metabolic rates will be examined and compared to tissue specific alterations in gene expression to help elucidate differences in adverse outcome pathways. In conclusion, acute aqueous PYR exposure resembles other AhR agonists, while NAP has additional cardiorespiratory toxic effects that make it a greater potential concern for acute sublethal toxicity in adult fish.

WP340 Phenanthrene may affect female reproduction via lipid homeostasis in fathead minnow liver

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Phenanthrene (PHE), a low molecular weight polycyclic aromatic hydrocarbon, is an environmental contaminant in waterways due to urban runoff, industrial effluents, and oil and gas extraction activities. Recently, PHE has been reported to affect reproductive processes in different fish species. The objective was to investigate the effects of PHE in both ovary and liver tissues to determine the potential impacts on the reproductive axis. Female fathead minnow (Pimephales promelas; FHM) were exposed to 0, 29.8, 389.4 and 942.5 µg/L average measured concentrations PHE over 24, 48 and 72 hrs. Ovarian tissues were processed for histology, ex vivo steroid production, and real-time PCR after 48hr exposure. There were no differences in steroid production or in the expression of transcripts involved in the steroidogenesis pathway. However, histological analyses showed increased proportions of cortical alveolar oocytes in 29.8 µg/L PHE fish and decreased proportions in 942.5 µg/L PHE fish suggesting that PHE may affect the distribution of oocyte stages in the ovary. As the liver is the main organ producing proteins for oocyte development, a transcriptomics experiment was performed to learn more about the pathways modulated in this tissue. Two microarray experiments were performed: a dose response at 48hrs exposure and a time course experiment over 72hrs at 29.8 µg/L. Sub-network enrichment analyses consistently identified pathways associated with lipids and fatty acids as affected by PHE. In the 48hr dose response experiment, pathways related to reproduction, lipids and fatty acids, and immune system function were affected. In the time course experiment, pathways affected included those related to reproduction, lipids and the immune system; there appeared to also be a shift towards lipid related processes at the later time point. In both experiments and across all treatments (dose and time), cholesterol metabolism was the one single pathway in common. We hypothesize that PHE may directly affect lipid processes in the liver, which leads to disrupted oocyte production downstream either through altered energy stores or lipid production.

WP341 Metabolic mechanisms of furan-based chemical alternatives (FBCAs): Assessment of toxicity at conserved aryl hydrocarbon receptors (AHR)?

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As trends toward urbanization and sustainable energy increase, biofuel consumption may become more widespread. There is a need to understand the resulting environmental consequences from the compounds found in greener fuel alternatives. Although legacy contaminants such as PCBs are decreasing, detection of biomarkers of aryl hydrocarbon receptor (AHR)-mediated toxicity (cytochrome P450 1A) is increasingly reported in fish from urbanized aquatic environments. Recent studies of chemical functional groups on aromatic hydrocarbons and their byproducts may differentially target AHR receptor pathways. We tested the hypothesis that furan-based biofuel analogs will differentially activate the zebrafish AHRs receptor and modify AHR targeted genes in early life stage zebrafish. Our screening approach consisted of developmental, cellular, and genomic endpoints. Zebrafish exposed at 24hpf were screened for 120hpf developmental endpoints. We selected the following furan-based compounds : a candidate biofuel, 2,5 dimethyl furan, and its combustion byproducts: 2,-3, hydrofuran, 2-ethylfuran, 2-methylfuran, 2-pentylfuran, 2,3-dimethylfuran, 2,5-dimethylfuran, furfural, indene, furan, as well as a potent AHR agonist, betanapthoflavone (BNF). Visualization of cyp1a-GFP was validated with qPCR of CYP1A1 gene expression. Characteristic AHR-dependent cardiotoxicity and pericardial edema measured for all compounds. Furfural, a dimethyl furan combustion product, produced characteristic cardiotoxicity. Using rt-qPCR, AHR battery genes nqo1, cyp1a1, nqo1, gst were measured for each chemical. The results in whole zebrafish were then compared to chemical-specific activation in invitro assays, and computational predictions of AHR activation with the furan library.

WP343 Bioaccumulation and toxicity of flame retardant TBPH or polychlorinated biphenyl PCB153 in dietary exposure in mummichog (Fundulus heteroclitus)

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The use of polybrominated diphenyl ethers as flame retardants in consumer products has been scrutinized increasingly due to their environmental persistence and potential toxicity; however, alternative replacement flame retardants may have similar drawbacks. The alternative brominated flame retardant bis(2-ethylhexyl)-2,3,4,5-tetrabromophthalate (TBPH) is a component of several commercial flame retardants, including Firemaster® 550, Firemaster® BZ-54 and DP-45. Here we investigate the bioaccumulation, bioenergetics and other adverse outcomes pathways (AOPs) predicted for dietary exposure to a carrier control, two levels of TBPH, or 2,2',4,4',5,5'-hexachlorobiphenyl (PCB153, a well-studied compound acting as a positive control for some aspects of the study). The TBPH concentrations chosen were at or well above the environmental concentrations documented in the literature, but similar to those causing toxicity in a previous study. Our experimental model is a small estuarine fish, the mummichog (Fundulus heteroclitus), exposed as individually tagged fish held in small groups (2 male, 2 female) in replicate tanks and fed contaminated food from day 0-28, followed by uncontaminated food from day 29-42. Throughout the experiment, individual growth was measured weekly, and at various time points, fish from replicate tanks were sacrificed, measured and dissected. To support putative AOPs, samples were obtained for analysis of hormone levels and transcriptomic responses to the exposures. After 28 days, the average sizes of fish in comparison to controls was larger in the PCB153 treatment group, and smaller in the treatment fed the high concentration of TBPH. These data will be used as input for a Dynamic Energy Budget model (currently under development for this species) to project effects throughout the life cycle. Rates of bioaccumulation and depuration determined during the study will be compared to predictions from standard bioaccumulation models, such as BASS. As expected, PCB153 was highly bioaccumulative and persistent; in contrast, a much smaller percentage of the provided TBPH was measured in fish. Coupling these growth and bioaccumulation data with ongoing transcriptomic analyses will improve our understanding of the bioaccumulation and adverse outcomes from dietary exposure to TBPH and PCB153. Ultimately, this work will support the application of ecological models to extrapolate effects of chemical exposures across species and chemical classes.

WP344 Tracking the fate of explosives, trinitrotoluene (TNT) and trinitrotriazine (RDX) in coastal marine ecosystems using stable isotopic tracer

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Although hundreds of coastal sites are contaminated with explosives, we know little about the fate of these explosives in coastal marine habitats. The goal of this study is to understand the fate of the explosives, trinitrotoluene (TNT) and trinitrotriazine (RDX) in coastal ecosystems using stable nitrogen isotopic tracer. Mesocosm experiments representing subtidal non-vegetated (coarse grained, organic-carbon-poor sediment and iron reducing conditions), subtidal vegetated and intertidal marsh (fine grained, organic-carbon-rich sediment and sulfate reducing conditions) ecosystems were conducted. Steady state concentrations of isotopically labelled ^{15}N-[TNT] and ^{15}N-[RDX] were maintained throughout a two-week period as two separate experiments. Sediment, biota, porewater and overlying water samples were analyzed for parent compounds, degradation products and ^{15}N inventories of inorganic nitrogen pools including ammonium, nitrate, nitrite, nitrous oxide and nitrogen gas. Quantification of sorption, degradation and mineralization using ^{15}N-isotopic tracer leads to mass balances of compounds in the systems. Fate of TNT and RDX in coastal marine ecosystems shows notable differences depending on characteristics of the compound. Persistence of TNT in mesocosms is fairly low compared to the RDX although the majority of system sequestrated TNT is unidentified. Higher sorption affinity of TNT and its derivatives makes sediment an effective sink with regard to remediation of contaminated aquatic environments. Instability of the aromatic ring of RDX facilitated ring breakdown and mineralization via several intermediates leaving nitrous oxide as the prominent product of RDX. Further, fate of TNT and RDX in coastal marine habitats depends on sediment characteristics and prevailing redox condition in the system. Subtidal vegetated and intertidal marsh ecosystems show notably higher mineralization rates for RDX in terms of production of dissolved inorganic nitrogen in the system. Sorption of TNT and derivatives onto sediment is highest in intertidal marsh system although no significant difference in mineralization among mesocosms. Sorption, biodegradation and mineralization of TNT and RDX were comprehensively related with geochemical variables of ecosystems using multivariate approach in order to acquire a better understanding of fate of compounds.

Neonicotinoid Insecticides: Current Research on Fate and Effects

RP001 Thiamethoxam concentrations in North American surface waters: A review of currently available data

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There has been increased attention related to neonicotinoid aquatic exposure and potential effects on aquatic invertebrate communities. Efforts have been made in peer-reviewed journal articles and non-peer-reviewed documents to summarize the current state of knowledge of neonicotinoid concentrations (as a class) in surface waters of North America. However, physicochemical properties such as water solubility and adsorption/desorption (Koc) properties as well as degradation via photolysis, aerobic/anaerobic metabolism and hydrolysis can vary widely among compounds within the class and can potentially influence their fate in aquatic habitats. The present work focuses specifically on thiamethoxam with the goal of compiling aqueous concentrations in systems which serve as potential habitats for aquatic invertebrates. We surveyed the peer-reviewed published literature and state and federal data sources for thiamethoxam monitoring data in aquatic systems in the U.S. and Canada. We only included sources in which individual sample data were published or individual sample data that were obtained from the study authors when only summarized data were presented. Data were obtained from monitoring studies across a spectrum of aquatic habitats including streams/rivers, prairie potholes, and playas. Based on currently available data, the majority of detections are at levels below established toxicity endpoints. These results will also be discussed in context of previously reported thiamethoxam aqueous concentrations and established effect data.

RP002 Determining occurrence of neonicotinoid insecticides in agricultural and urban impacted US streams

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To better understand the fate and transport of neonicotinoid insecticides, water samples were collected from streams across the United States. In a nationwide study of 38 streams, at least one neonicotinoid was detected in 53% of the samples collected, with imidacloprid detected most frequently (37%), followed by clothianidin (24%), thiamethoxam (21%), dinotefuran (13%), and acetamiprid (3%). Clothianidin and thiamethoxam concentrations were positively correlated to the percentage of the land use in cultivated crop production and imidacloprid concentrations were positively related to the percentage of urban area within the basin. Additional sampling for neonicotinoid insecticides was also conducted in localized research areas to complement the national-scale results, including: (1) basins which have both agricultural and urban land uses (California, Georgia, Great Lakes Region), and (2) predominantly agricultural basins in two nationally important ecosystems (Chesapeake Bay and San Francisco Bay-Delta). These environmental data are important in determining the potential risk of neonicotinoids to non-target aquatic and terrestrial organisms.

RP003 Characterization of acephate and imidacloprid loss in containerized nursery runoff water

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Pesticide formulation, application method, and irrigation intensity may significantly affect the losses of pesticides found in surface runoff water from containerized plant nurseries. For this study, four pesticides are being evaluated. Granular and sprayable formulations of acephate, bifenthrin, chlorpyrifos, and imidacloprid are being evaluated in small-scale plots irrigated at three different intensities. Plots were constructed in a shade house on the University of Florida campus in Gainesville, FL. Collection trays lined with plastic and landscape fabric were designed to collect drainage water into composite containers from individual containerized plants. Total mass (ug) of each pesticide discharged is calculated by multiplying the concentration (ug/L) by the volume discharged (L). Percentage of pesticide loss is determined by dividing the total mass discharged by total mass applied. In preliminary studies, containerized 1 gal dwarf Burford holly were treated with per pot applications of Precise 4% G (acephate; solubility: 7.9 x 10^5 mg/L at 20°C) and Marathon 1% G (imidacloprid; solubility: 6.1 x 10^2 mg/L at 20°C) at rates of approximately 15 g/pot and 5 g/pot , respectively. 14.5 L of water was applied daily for 10 days during 23 minute overhead irrigation cycles and all runoff water was collected in amber glass bottles. Total mass of acephate lost (n=3) was 1899 ± 1358 µg, with a percentage loss of 0.30 ± 0.22%. Acephate concentrations ranged from 3 µg/L to 1265 µg/L. The total mass of imidacloprid lost (n=3) was 6892 ± 262 µg with a percentage loss of 12.2 ± 0.73% during the same irrigation events. Pesticide losses relative to rainfall intensity and method of application (sprayed vs. per pot) will be discussed in this presentation.

RP004 Mass Balance Assessment for Neonicotinoids During Wastewater Treatment and Nationwide Occurrence in United States Wastewater

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Over the last decade, an increased usage has been noted for neonicotinoids – a group of systemic insecticides with applications in agriculture, horticulture, and for urban nuisance pest control including pet flea treatment. Yet, knowledge is still limited as to the occurrence and fate of various neonicotinoid pesticides in urban wastewater process flows. Goals of the present study were to: (i) conduct a mass balance assessing the fate of six neonicotinoids during conventional wastewater treatment; (ii) assess the efficacy of wetland treatment; (iii) obtain the first national emission estimate by monitoring additional 12 treatment facility from across the United States; and (iv) to perform the first regional study in California via monitoring at eight local facilities. A mass balance based on analysis of flow-weighted 24-hour composites revealed insignificant removal of imidacloprid (p = 0.09, CI = 95%) and limited removal of the sum of acetamiprid and its primary degradate, acetamiprid-N-desmethyl (18 ± 4%, p = 0.01, CI = 95%). In the wetland, no removal of imidacloprid or acetamiprid were observed. Nationwide, imidacloprid, acetamiprid and clothianidin occurred at ng/L concentrations in WWTP influent (60.5 ± 40.0; 2.9 ± 1.9; 149.7 ± 289.5) and effluent (58.5 ± 29.1; 2.3 ± 1.4; 70.2 ± 121.8). Clothianidin was found only intermittently, whereas thiamethoxam, thiacloprid, and dinotefuran were never detected. Extrapolation of data from 13 WWTPs to the nation as a whole suggests annual discharges on the order of 1000-3400 kg/y of imidacloprid contained in treated effluent to surface waters nationwide. In eight Northern California WWTPs sampled under low-flow drought conditions, imidacloprid, which is used indoors primarily for pet flea treatment, was detected with 100% detection frequency (DF) in influent and effluent samples at 58-306 ng/L, concentrations that were generally higher than those observed nationwide. Sludge analysis yielded no detections (< 5 ng/g dw). Ubiquitous detection of neonicotinoids in wastewater demonstrates continuous entry of these compounds into the urban water cycle and their pronounced persistence during wastewater and wetland treatment, enabling entry into surface waters at significant loadings that potentially are harmful to sensitive aquatic invertebrates.

RP005 Transformation and fate of neonicotinoid insecticides during drinking water treatment

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Despite their widespread detection in surface waters, relatively little is known about the fate of neonicotinoid insecticides during engineered water and wastewater treatment. This work explores the transformation and removal of common neonicotinoids via the physical and chemical processes used in conventional drinking water treatment. The goals of this work are to establish the rates and extent of neonicotinoid transformation and/or removal during simulated treatment operations in the laboratory, while also identifying major transformation products generated during chemical treatment processes. In ongoing work, occurrence studies are seeking to identify transformation products from laboratory experiments and in finished drinking water. The results of this work will help to better identify potential risks posed to humans and other non-target organisms by neonicotinoids and their transformation products generated during treatment.

RP006 Investigating the Cumulative Toxicity of Neonicotinoid Insecticide Mixtures to Chironomus dilutus using MIXTOX Analysis

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Neonicotinoids are a popular class of systemic insecticidal treatment, commonly applied as a seed treatment to protect young crops against biting-sucking insects. Extensive use of these seed treatments (e.g., imidacloprid, clothianidin, and thiamethoxam) in Canadian Prairie agricultural regions has resulted in the detection of neonicotinoid mixtures in ecologically important wetlands surrounding these arable areas. However, the impact of these mixtures on local insect communities is poorly understood. This research aims to address that knowledge gap by characterizing the cumulative toxicity of binary and tertiary mixtures of select neonicotinoids (imidacloprid (IMI), clothianidin (CLO), and thiamethoxam (TMX)) to the sensitive aquatic insect Chironomus dilutus under acute (96 h) exposure scenarios. Single-compound toxicity bioassays yielded toxicity threshold values (LC_{50}) of 4.63 (3.96 – 5.41), 5.93 (5.29 – 6.65), and 55.34 (43.98 – 69.64) for IMI, CLO, and TMX, respectively. These values were used to develop parametric models, which were statistically compared to the toxicity of binary and ternary mixtures from similar laboratory studies using the MIXTOX approach. CLO-TMX mixtures demonstrated concentration-additive synergistic toxicity. IMI-CLO mixtures demonstrated response-additive synergistic toxicity. IMI-TMX mixtures demonstrated response-additive dose-ratio dependent synergism, with toxicity shifting from antagonism to synergism as the relative concentration of TMX was increased. IMI-CLO-TMX mixtures demonstrated response-additive synergistic toxicity. Results obtained indicate that under acute exposure scenarios, the toxicity of these neonicotinoid mixtures to sensitive aquatic insects cannot be adequately predicted by direct addition of single compound concentrations. These results will be compared to those garnered in concurrent chronic (28 d) mixture studies, allowing for an assessment of whether these insecticide mixtures display similar deviations from concentration addition under more environmentally realistic exposure paradigms, and what risk this may pose to the aquatic environment.

RP007 Assessing the acute and chronic toxicity of neonicotinoid insecticides to non-target aquatic species

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Neonicotinoids are the most widely used insecticides in the world. They are preferentially toxic to insects while displaying a low toxicity toward vertebrates; this selective toxicity has led to their rapid and ubiquitous use. However, neonicotinoids may negatively affect aquatic ecosystems because they are environmentally persistent and highly water-soluble, and thus are prone to leaching from the soil into surface waters via run-off events. Although non-target aquatic organisms may be adversely affected by these compounds, few data are available regarding the effects of neonicotinoids on aquatic invertebrates. The objective of this study was to assess the acute and chronic toxicity of six neonicotinoids (imidacloprid, thiamethoxam, acetamiprid, clothianidin, thiacloprid, and dinotefuran) to Hyalella azteca (amphipod) and Hexagenia spp. (mayfly). Neonicotinoid exposures were conducted in water-only systems, and test durations were 28 d (amphipod) and 96 h (mayfly). Endpoints assessed included survival and growth in amphipod tests, and survival and behaviour (number of animals inhabiting artificial burrows) in mayfly tests. Effects of neonicotinoids on amphipods varied depending on the compound: 28-d LC50s were 3, 4, 40, 50, 90, and 200 µg/L for clothianidin, acetamiprid, dinotefuran, thiacloprid, imidacloprid, and thiamethoxam, respectively. Amphipod growth was reduced at lower concentrations than survival for imidacloprid and thiacloprid, with 28-d EC50s of 4 and 3 µg/L, respectively. Effects of neonicotinoids on mayflies were variable among compounds: 96-h LC50s were 600, 700, 800, and 3000 µg/L for imidacloprid, clothianidin, acetamiprid, and thiacloprid, respectively, and were > 10,000 µg/L for thiamethoxam and dinotefuran. However, mayfly behaviour was inhibited at lower concentrations than survival for all neonicotinoids, with 96-h EC50s for number of animals inhabiting artificial burrows of 4, 4, 10, 20, 20, and 200 µg/L for acetamiprid, thiacloprid, imidacloprid, clothianidin, dinotefuran, and thiamethoxam, respectively. In addition, mayfly mobility was inhibited by imidacloprid, acetamiprid, and thiacloprid at concentrations ≥ 1 µg/L. The maximum environmental concentration of neonicotinoids measured in 2012-2014 surveys of 16 stream sites in southern Ontario was imidacloprid at 10 µg/L, indicating that environmental concentrations may adversely affect sensitive aquatic species.

RP008 Early chironomid emergence from chronic, low-level neonicotinoid exposure in a prairie pond: Is timing everything?

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Seasonal timing and phenological synchrony are critical components to an aquatic insect lifecycle and long-term population viability. Immature aquatic insects occupying surface water bodies adjacent to agricultural operations planted with seed-treated neonicotinoids are susceptible to chronic exposure from repeated run-off and long-term environmental persistence. These scenarios are often incredibly difficult to replicate with any level of control to determine environmental risk. To mimic these conditions, an in-situ, semi-controlled limnocorral experiment compared the neonicotinoids: imidacloprid (IMI), clothianidin (CLO), and thiamethoxam (THX) at two doses, 0.05 (low) and 0.5 (high) µg/L. Over a 15-week study, 21 limnocorrals were dosed weekly for 9 weeks to achieve a chronic, repeated exposure scenario simulating repeated run-off conditions, followed by a 6-week recovery period. Water concentrations and aquatic insect emergence were monitored continuously throughout the study. Thirty families and four chironomid tribes were identified over all treatments with two taxa exclusively emerging from the control treatments, (Hymenoptera: Trichogrammatidae) and (Diptera: Tipulidae). The Chironomidae was the most abundant family, representing 65% of all production. Total abundance of emerged chironomid adults among treatments were not significantly different (One-way ANOVA; F=0.752, df=6, n=21, p=0.618). However, on average the cumulative proportion emerged was 21-24 days, 7-18 days, and 3-16 days earlier than the control limnocorrals for imidacloprid, clothianidin, and thiamethoxam limnocorrals, respectively. Given the relatively short life span of adults among different taxa groups, difficulties of finding a mate in a fragmented landscape, and use of insect secondary production as a food source by higher trophic organisms, these expedited emergence events may have direct and indirect implications to aquatic insect communities and wetland dependent fauna, alike. Results from this study merits further investigation on the potential effect neonicotinoids may have on insect hormone regulation.

RP009 Effects of chronic exposure to thiamethoxam on summer generation mayfly populations in an outdoor mesocosm

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The effect of neonicotinoids on mayfly nymphs has been investigated in recent literature, demonstrating they are amongst the most sensitive aquatic insect taxa to these insecticides. A publication by van den Brink et al (2015) reported the chronic effects of a continuous thiamethoxam exposure (28d) under laboratory conditions on over-wintering generation mayfly larvae (Cloeon dipterum); 28d EC_{10} = 0.43 ug a.s./L. A GLP outdoor mesocosm study was performed to investigate chronic effects of a continuous exposure to thiamethoxam on a summer generation mayfly (Cloeon dipterum) population. Twenty stainless steel enclosures within one large pond enclosure were used for the study; five untreated controls and five treatment levels with three replicates each. The treatment levels were, 0.1, 0.3, 1.0, 3.0, 10 µg a.s./L. Concentrations of thiamethoxam were maintained with twice-weekly applications to the water column via mixing. The time weighted average concentrations ranged from 93 to 108% of nominal, with a mean of 101% of nominal. Mayfly abundance was assessed with sweep-netting and substrate sampling (two baskets with aquatic macrophytes and stones). Adult emergence was also sampled. Mayfly sampling occurred weekly for the duration of the study. Statistical analysis (method: Minimum Detectable Difference (MDD);Brock et al, 2015) indicated that small effects could be detected throughout the study duration, with one exception. On day 34, only large effects could be detected, due to natural population decline. The no-observed-effect-concentration (NOEC) for larval abundance was determined to be 0.3 µg a.s./L and the lowest-observed-effect-concentration (LOEC) was determined to be 1.0 µg a.s./L.The results for adult emergence support the observed effects on the larval population. The results of the study indicate that under conditions of continuous exposure, there is no apparent difference in sensitivity to thiamethoxam between summer generation mayfly larvae tested under field conditions and over-wintering generation mayfly larvae that were tested under laboratory conditions. This study adds to the debate on the effects of neonicotinoids on sensitive aquatic insects by determining a no effect level for mayfly populations in outdoor mesocosms continuously exposed to thiamethoxam, representing worst-case conditions with respect to the EU FOCUS modelling drainage scenarios.

RP010 Residues of thiamethoxam at early reproductive stages of soybean and its potential impacts on non-target insects

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Thiamethoxam is a widely used neonicotinoid compound applied as a seed treatment in soybean crops. During the last few years there has been a growing concern about the impact of thiamethoxam on beneficial insects in soybean. Negative impacts on beneficial arthropod communities are based on the hypothesis that neonicotinoid residues can be present in soybean vegetative tissue, host insects and flower tissues making them toxic to pollinators and natural enemies. The risk characterization of neonicotinoids to natural enemies in soybean requires baseline information of the concentration in soybean vegetative and floral tissue and the toxicity of this compound on beneficial insects associated to this crop through multiple routes of exposure to the insecticide. To identify exposure levels of non-targets insects in soybean fields we evaluated the translocation of thiamethoxam and mefenoxam applied as seed treatments in soybean crops. Furthermore, we evaluated the toxicity of thiamethoxam on key natural enemies of soybean aphid exposed to residues in vegetative tissue and in insect prey. To achieve this objective, the predators Orius insidiosus and Crysoperla rufilabris were exposed to different concentrations of thiamethoxam in soybean leaves and in soybean aphid using different laboratory methodologies. The results showed that the concentrations required to kill more than 50% of the evaluated insects were higher than the concentrations that the insects are likely to encounter in the field. There was found that the concentration-response evaluation of predators needs to be developed before 24 h if there is no availability of an alternative food source. Consumption of soybean aphid by O. insidiosus and C. rufilabris was not significantly affected at evaluated concentrations of thiamethoxam at 24 hours of evaluation. However significant mortality was observed in O. insidiosus at 24 hours after exposure to thiamethoxam treated aphids. The main challenges to determine the impact of neonicotinoids in soybean crops on non-target insects will be discussed.

RP011 Effect of Two Neonicotinoid Pesticide Products upon Utilization of Milkweed Plants by Monarch Butterflies

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Monarch butterfly larvae feed exclusively on milkweed plants (Asclepias spp). Comprised of over 100 different species in North America, milkweed plants grow in many locations including within and near agricultural lands in the Midwestern United States where they are considered pest plants, and are planted in residential flower beds due to their beauty and the tendency of their flowers to attract butterflies. In both situations, monarch larvae may be exposed to pesticides. Populations of monarch butterflies have declined over the past 10 – 15 years due to a variety of possible factors including habitat loss (i.e., loss of host milkweed) and pesticides. The study underway is evaluating monarch utilization of milkweed plants potentially contaminated by neonicotinoid pesticides from two different pesticide products (granular and corn seed treatment). For the seed treatment (active ingredient [a.i.] thiamethoxam), 200-gallon tubs with established milkweed plants were planted with treated (10 tubs) or untreated (10 tubs) corn seed in the spring and monitored weekly for utilization by monarch butterflies (number of eggs, larvae, and chrysalises). Corn and milkweed leaves, soil, and leachate were sampled 4-to-6 weeks after planting, and analyzed for thiamethoxam. For the granular product (a.i. clothianidin), 200-gallon tubs with newly planted milkweed were treated with the granular product at the label rate as well as at a medium (60 percent of label rate) and low rate (60 percent of medium) plus a control (3 tubs). Monarch utilization of the milkweed in the granular treatment tubs were monitored weekly for 6 months. Based on the label recommendations for the granular product, the tubs were retreated with every 8 weeks. Milkweed leaves, soil, and leachate were sampled following 5 months of treatments and analyzed for clothianidin. This study will provide information on the possible effect upon monarch reproduction of pesticide products with neonicotinoids. It will also provide data on the fate and transport of the respective neonicotinoids from the source (treated seed or granules) to soil, leachate, and lastly milkweed foliage that the monarch larvae consume.

RP012 Effects of imidacloprid and chlorpyrifos insecticides on migratory behaviour and physiology in a passerine, the white-crowned sparrow

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Many migratory birds utilize agricultural landscapes for refuelling where they may be exposed to current-use insecticides through consumption of treated seeds, granules, or sprayed soils. Numerous insecticides are known neurotoxins, which could cause changes in critical phases of avian migration, such as mass gain, cognition and orientation behaviour. Changes in migratory behaviour could affect the ability of birds to successfully reach the breeding or wintering grounds, and ultimately have long-term effects on reproduction and survival. However, little is known about the direct impacts on migration. We compared behavioural and physiological effects and recovery following acute exposure to sublethal levels of two pesticides with different modes of action, Imidacloprid (IMI; a neonicotinoid) or Chlorpyrifos (CPF; an organophosphate), in a migratory seed-eating bird, the white-crowned sparrow. Sparrows were captured in the spring at migratory stopover sites in Saskatoon, Saskatchewan, and taken into captivity. Migratory behaviour was monitored with Emlen funnels, which can be used to assess migratory orientation and activity levels. After an acclimation period of ~2 weeks, birds were pre-tested in Emlen funnels to confirm they were in a state of zugunruhe (migratory restlessness). They were then dosed daily for 3 days with either the vehicle control (sunflower oil) (n = 10), 4.1 mg IMI/kg (n = 11), 10.3 mg IMI/kg (n = 11), 2.9 mg CPF/kg (n = 9) or 7.4 mg CPF/kg (n = 9), representing an estimated 10% and 25% of the LD50. Birds were re-tested in Emlen funnels at the end of dosing, after a 3-day recovery period, and again after a 14-day recovery period. Mass and fat was scored throughout the experiment. Once 14-day recovery trials were complete, birds were euthanized and livers and brains were collected for future analysis. Following IMI exposure, we observed acute effects in both exposed groups, including significant mass loss, reduced foraging, excess saliva in the crop, and respiratory distress. In birds exposed to CPF, we observed moderate weight loss in the high dose group, but no other overt effects. Effects on migratory activity, orientation behaviour, and recovery, are currently being assessed. This is the first study to evaluate the direct toxicity of neonicotinoids in comparison with older organophosphate chemistries. Uniquely, we were able to assess the sublethal effects on behaviour and physiology in wild songbirds during a migration event.

RP013 Impacts of passive contaminant exposures, including imidacloprid, on parental care behaviors and testosterone of golf course living eastern bluebirds

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Parental care behaviors, such as nest building, incubation, and nestling provisioning are essential for nest survival and fledging in monogamous birds. Such behaviors often correlate with ornamental plumages or physiology in many species. Expression of these traits may be influenced by genetics, environment, or hormones. Recently, behavioral studies demonstrate that anthropogenic disturbance can lead to altered parental care. Examination of behavior in relation to human disturbance and environmental contamination is increasing given relevant impacts behavior has on fitness in wildlife populations. Research presented here provides partial support for the hypothesis that level of human disturbance (including indirect, passive contaminant exposures) influence parental care behaviors in eastern bluebirds at a chronically disturbed golf course compared to those breeding at a rural military base. Sites differ significantly in habitat, human activity, and pesticide use, and golf course birds are more ornamented. Consistent with predictions, golf course pairs complete nests more slowly, produce smaller clutches, and fledge fewer young. Contrary to predictions, incubation length, and male feeding rate does not differ between sites. Female feeding rates, however, differ between sites but not in the direction predicted; golf course females provision at higher rates. Lastly, during nestling provisioning, golf course male display lower T while females display slightly lower T than birds at the rural site, and female T is positively correlated with higher average feeding trips (regardless of nestling number). There are no relationships between ornamentation and parental behaviors or reproductive indices, contrary to evidence from other bluebird populations. Lastly, males were observed performing female-typical nesting and incubation behaviors, and although not quantified, this observation appears unique for males of this species. While contaminants were not quantified in this research, data suggests that factors contributing to anthropogenic disturbance may cause females to delay breeding and reduce clutch investment but, later in the nesting cycle, does not appear to negatively influence provisioning behaviors. Delays in female breeding may also result in male compensation of previously unperformed parental behaviors to increase individual or pair fitness.

RP014 Northern leopard frog (Lithobates pipiens) developmental responses to neonicotinoid exposure: preliminary findings

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In recent years, neonicotinoid use has been linked to collapses of honey bee (Apis mellifera) colonies; however, relatively little is known about the impact of neonicotinoid insecticides on vertebrate wildlife. Amphibians are sensitive to environmental stressors and may be more vulnerable to neonicotinoid exposure due to their dual aquatic/terrestrial life cycle. Consequently, amphibians are excellent vertebrate bioindicators for a given environment. The objective of our study was to characterize the sub-lethal effect(s) of exposure to ecologically-relevant levels of neonicotinoid insecticides on northern leopard frogs (Lithobates pipiens, formerly Rana pipiens) during their aquatic life-cycle stage using mesocosm and laboratory exposures. Our previous work (summer, 2015), exposed northern leopard frog tadpoles to formulated neonicotinoid products containing thiamethoxam or clothianidin as the active ingredient in outdoor mesocosms. The exposure resulted in no effects on survival, growth or development. Here we followed up on our previous work via laboratory exposures with the technical product clothianidin to separate potential indirect and direct effects of neonicotinoids on tadpoles. In May of 2016, adult northern leopard frogs were bred in outdoor mesocosms and egg clutches were allowed to develop. Tadpoles from Gosner stage 25 to 42 were exposed for 8 weeks to environmentally-relevant concentrations (0 to 100 µg/L) following a static-renewal system. We assessed the effects of chronic exposure to clothianidin on conventional tadpole life-history traits, such as survival, growth and development that can affect amphibian fitness. Another objective was to assess whether neonicotinoid exposure affected tadpole susceptibility to parasitic helminths to add a disease-related component. The results of this study will help in establishing water quality guidelines and regulations for neonicotinoid use in Canada; however, more information is required on monitoring environmental concentrations of neonicotinoids and sub-lethal effects on chronically exposed non-target organisms to fully understand the risk of neonicotinoids on ecosystems.

RP015 Sub-lethal effects of the neonicotinoid clothianidin on developing sockeye salmon (Oncorhynchus nerka)

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Current-use pesticides are a concern for the health of anadromous salmonids throughout the Fraser River Basin in British Columbia, Canada. Neonicotinoids are a class of widely used insecticides that have been detected in ng-µg/L concentrations in surface waters globally, including those in the Fraser River Basin, yet few studies have examined the potential sub-lethal effects of neonicotinoids on aquatic vertebrates. This study examined the effects of a neonicotinoid insecticide, clothianidin, following a chronic exposure, on critical early life stages of a wild salmon species, sockeye salmon (Oncorhynchus nerka). In this study, 4 concentrations of clothianidin (0.15, 1.5, 15 and 150 µg/L) plus a water control were tested in a chronic exposure experiment that was initiated immediately post-fertilization and experiment continued through to the swim up fry developmental stage. Three unique offspring sets (crosses) were tested within each experiment and all treatments were conducted in duplicate. Clothianidin did not affect survival or morphometrics in chronic exposure, although differences between crosses within clothianidin treatments were evident. Gene expression analyses to examine alterations in hepatic endocrine, nervous and immune system transcript abundance and hormone concentration analyses are ongoing to further investigate the sub-lethal effects of clothianidin in early life stage sockeye salmon. Supported by Fisheries and Oceans Canada National Contaminants Advisory Group.

RP016 Sublethal doses of imidacloprid reduce the efficacy of visual collision detection in Locusta migratoria

R. Parkinson, J.R. Gray, Univ of Saskatchewan / Biology

Imidacloprid is known to be directly toxic to insects and aquatic invertebrates at agriculturally-relevant doses. Indirect toxicity also occurs bees, birds, and other non-target organisms. Locusts (Locusta migratoria) are persistent and important agricultural pests, and once mature can survive high pesticide doses. We show that sublethal doses of imidacloprid disrupt the neuronal processing of salient visual cues, including those of objects approaching on a direct collision course. The Descending Contralateral Movement Detector (DCMD) is a motion sensitive neuron in the locust brain that is involved in collision avoidance and escape from predators. Analogous neurons exist in many species, including pigeons, crabs, and other insects such as flies. Typical responses of the locust DCMD to looming stimuli are well documented, providing a unique substrate for examining real-time effects of the neonicotinoid pesticide on a well-described visual detection system. Locusts were tested with sublethal doses of imidacloprid incrementing by an order of magnitude from 0 ng g^{-1} to 100 ng g^{-1}, with significant effects resulting from both 10 and 100 ng g^{-1} doses. Alterations of the firing properties of the DCMD were sustained even after acute effects of the dose subsided, suggesting the pesticide is capable of causing lasting changes to the neurons in this pathway. A dose response curve illustrated the relative effects within the range of doses tested. We show that sublethal doses may be indirectly lethal to mature locusts by disrupting a key motion detection pathway. This research is relevant to other insect species, including important pollinators, that may use analogous collision detection systems.

RP017 Sublethal concentrations of thiamethoxam affect synapsin levels in mushroom bodies of Apis mellifera after exposure in the larval period

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The exposure of bees to neonicotinoid insecticides such as thiamethoxam, has been appointed as one of the factors responsible for the worldwide decline of populations of Apis mellifera. Because having systemic action in the plant, this insecticide can contaminate the pollen, nectar and other floral resources used by bees as food. Thus, in addition to the exposure of worker bees during their forager activity on field, the bees that remain inside the hive can also be exposed to thiamethoxam through the consumption of contaminated pollen and nectar that have been stocked inside the colony, which are also provided to larvae by the nursing workers. The neonicotinoids are neurotoxic insecticides, which act as agonists at nicotinic acetylcholine receptors of the nervous system of insects, especially in the brain. Its molecules bind irreversibly changing the pattern of nerve transmission and causing excitation, paralysis and eventual death of the insect. This study aimed to analyze the immunostaining of synapsin in the brain, specifically in the pedunculated bodies, because this protein is involved in the process of releasing of acetylcholine vesicles during the neural synapses. In order to assess the neurotoxicity of thiamethoxam to pupae and newly emerged workers of Africanized Apis mellifera, the larvae were previously exposed to three sublethal concentrations of thiamethoxam, as follows 0.00001 ng/µL; 0.001 ng/µL e 1.44 ng/µL in the diet (acute oral exposure). Sections of bee brains were performed in a vibratome and the sinapsin immunostaining was detected by confocal laser scanning microscopy. The results showed a significant decrease in immunostaining of synapsin in the pupae brain from the group exposed to 0.001 and 1.44 ng/µL, as well as a decrease of this labelling in the brain of newly emerged workers from the group exposed to 1.44 ng/µL in comparison to the control group. The results demonstrate that exposure to sublethal concentrations of thiamethoxam during the larval stage may cause impairment of neural functions performed by the brain pedunculated bodies in pupae and newly emerged workers, which may negatively affect the performance or the survival of bees.

RP018 Imidacloprid promotes genetic damage and oxidative stress in different tissues of the Neotropical fish Prochilodus lineatus

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Imidacloprid (IMI) is a systemic neonicotinoid insecticide widely used around the world, and recent data highlight this compound among the ten active ingredients commercialized in Brazil. However, despite its increasing use only few toxicity studies have been performed about its effects on non-target organisms. The aim of the study was to identify the potential hazard of the commercial formulation Imidacloprid® (48% imidacloprid- Nortox S.A. Brazil) in juveniles of Prochilodus lineatus (14.1 ± 1.14 g, 10.77 ± 0.6 cm [mean ± SD, n=40]). Fish were exposed to IMI at nominal concentrations of 5 (IMI5), 50 (IMI50), 500 (IMI500) and 5000 (IMI5000) µg.L^{-1} or only to clean water (CTR) for 120 h, under static condition and daily water renewal. Biochemical parameters such as glutathione content (GSH), activity of the glutathione-S-transferase (GST) and acetylcholinesterase (AChE), lipoperoxidation (LPO), protein carbonylation (PC), genotoxic biomarkers (DNA damage), micronuclei (MN) and the erythrocytic nuclear abnormalities (ENAs) were evaluated in various organs. When compared to CTR fish, significant increases of LPO and PC were observed in the liver of fish exposed to IMI5000. In the gills we observed a significant increase of LPO in fish exposed to IMI50 and IMI500, and an increase of PC together with reduced GST activity in fish exposed to IMI5000. Fish exposed to IMI500 and IMI5000 showed significant increases of PC in the kidney. In addition, an increase of LPO and decrease of GST activity were observed in the kidney of fish from IMI5000 group. In the brain, a raise of GSH with concomitant increase of GST activity was observed at IMI500 and IMI5000. LPO also increased in the brain of fish exposed to IMI5 and IMI50, suggesting that the activation of antioxidant defenses (GSH and GST), observed at higher concentrations prevented oxidative stress in this organ. The comet assay showed increase DNA damage ($p = 0{,}018$) in the erythrocytes of fish exposed in all IMI concentrations tested. Also, there was a significant increase in ENAS frequency in fish exposed to IMI5000 ($p= 0{,}043$), relative to others groups. Taking together, these results suggest that IMI has the potential to promote oxidative stress and genotoxicity in P. lineatus, and kidney and gills proved to be the most affected organs, followed by the brain which showed oxidative damage in fish exposed to the lower IMI concentrations, which are the most environmentally relevant.

RP019 Weight-of-evidence evaluation of an adverse outcome pathway network linking activation of the nicotinic acetylcholine receptor in bees to colony loss

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Ongoing honey bee colony losses are of significant international concern because of the essential role these insects play in pollinating staple food crops. Chemical and non-chemical stressors both have been implicated as possible contributors to colony failure, however, the potential role of commonly-used neonicotinoid insecticides has emerged as a focal point of potential chemical stressor impacts. Neonicotinoids act on the nicotinic acetylcholine receptor (nAChR) to eliminate target pest insects. However, mounting evidence indicates that these chemicals may adversely affect beneficial pollinators, such as the honey bee, through multiple mechanisms including impacts on learning and memory thereby affecting foraging success. However, the mechanisms linking activation of the nAChR to adverse effects on learning and memory are uncertain. Additionally, clear relationships between observed impacts on individual bees and colony level effects are lacking. Therefore, the objective of this work was to develop adverse outcome pathways (AOPs) as a means to evaluate the biological plausibility and empirical evidence supporting (or refuting) the linkage between the nAChR and colony level impacts. Development of these AOPs has led to the identification of research gaps which, for example, may be of high priority in understanding how perturbation of pathways involved in neurotransmission can adversely affect honey bee health, causing colony instability and failure. From this effort, an AOP network also was developed, laying the foundation for further insights as to the role of combined chemical and non-chemical stressors in impacting bee populations. Insights gained from AOP network assembly, which more realistically represent multi-stressor impacts on honey bee colonies, are promising aids for understanding common nodes in the biological pathway and identifying where mitigation strategies may be focused to reduce colony losses. The contents of this abstract neither constitute nor reflect official USEPA policy.

RP020 Quantitative Weight of Evidence Assessment of Higher Tier Studies on the Toxicity and Risks of Imidacloprid in Honeybees: I Methods & Procedures

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Higher tier studies have been conducted on the toxicity of neonicotinoids to honeybees, some in the open literature and some by the registrants of these insecticides. Individually, these studies were of varying quality; collectively, they comprise a body of evidence of the potential effects of these chemicals under more realistic conditions than those in laboratory studies. A framework for a quantitative weight of evidence methodology (QWoE) was developed and used to assess the effects of imidacloprid (IMD) on honeybees. Assessment endpoints were population size and sustainability of commercially managed bees via quantity and quality of hive products. A conceptual model for exposures was used to characterize important pathways of exposure of honeybees. Nectar and honey were potentially the most important sources of exposure for worker bees. Surface waters and guttation fluid from plants grown from treated seed was an incomplete pathway for all stages and casts. The queen is protected from direct exposures and larvae to a lesser but significant extent. The QWoE was based on a scoring system for evaluating the reports provided by the registrant and papers from the open literature. Criteria for assessing quality and relevance were developed before the assessment began and were based on best practices in ecotoxicology for measuring responses at the level of the hive and exposures via bee-relevant matrices such as nectar, pollen, honey, water, guttation fluid and dust. Some scoring criteria were critical (each had their own score) and others were general. General aspects of the experiment and the analysis of the data were grouped and weaknesses noted and used in the scoring. Scores for relevance were based, inter alia, on statistical significance and threshold no-observed-adverse-effect doses from toxicity tests at the level of the hive. Scores were assigned on a relative scale of 0-4 using a formal guide for scoring. The scores were averaged and then used to separate the higher from the lower quality studies and the relevant from the non-relevant results. The basis for each score was documented in a narrative and this was subjected to independent quality assurance. These scores were summarized graphically to illustrate the quality of the studies and their relevance and thus the overall strength of the evidence. Assessment endpoints were population size and sustainability of commercially managed bees via quantity and quality of hive products.

RP021 Quantitative Weight of Evidence Assessment of Higher Tier Studies on the Toxicity and Risks of Imidacloprid in Honeybees: II Results & Conclusions

G.L. Stephenson, Aquaterra Environmental Consulting Inc. / Environmental Services; K.R. Solomon, Univ of Guelph / School of Environmental Sciences

A quantitative weight-of-evidence (QWoE) analysis was applied to field and semi-field studies designed to assess the effects of the neonicotinoid, imidacloprid (IMD) on honeybees. The analysis considered the potential risks associated with exposure of honeybees to IMD via residues in bee-relevant matrices (nectar, pollen, guttation fluid, bee bread) and via different types of applications (seed dressing, foliar, soil drench, artificial feeding, and dust). The QWoE analysis data for exposures via IMD-dressed seed indicated that environmentally realistic concentrations (< 20 µg IMD/kg) result in no adverse effects to apical endpoints of honeybee colonies. The quality of the methods (QoM) or data (n=85 endpoints) was variable and relevance low (QoM = 1.21 ± SE of 0.06, relevance = 0). When applied to semi-field feeding studies, the QWoE analysis of dietary exposures (IMD-amended syrup or pollen patties) indicated that honeybees are at risk only when exposed to IMD at environmentally unrealistic concentrations >20 µg/L or kg. The quality of the studies and data (n=21 endpoints) was relatively strong and relevance low (QoM = 2.28 ± SE of 0.25, relevance = 0.2). The impact of dust residues on foraging honeybees and those flying in adjacent fields with flowering plants was negligible (QoM = 2.28 ± SE of 0.25) when the route of exposure considered was pollen and nectar originating from plants where TMX was taken up by plants. Non-seed applications of IMD (foliar, dust, and soil drench applications) were evaluated and the QWoE analysis indicated that the quality of the studies and data (n=28 endpoints) was variable and the relevance low (QoM = 1.64 ± 0.0, relevance = 0). One study indicated that a combination of concurrent foliar and soil applications could result in potentially hazardous nectar and pollen exposure concentrations; however, the QWoE for effects indicated no adverse effects. For higher-tier studies (ecoepidemiological), the weight of evidence did not support a causal relationship between exposure to IMD and adverse effects to honeybees. The data (n=32 endpoints) were from studies that varied in quality (QoM = 1.88 ± SE of 0.19; relevance 0.11 ± SE of 0.11); however, both the stronger studies and the weaker studies (with one exception) consistently showed no adverse effects. The overall weight of evidence suggests that there is minimal risk to honeybees at the colony level from exposure to IMD used as a seed treatment.

RP022 Quantifying sources of variability in neonicotinoid residue data for assessing risks to bees

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The U.S. Environmental Protection Agency's 2014 guidance for assessing pesticide risks to bees relies on higher-tier studies of residues in pollen and nectar to refine pesticide exposure estimates obtained from lower tier information (e.g., default values and models). These higher tier residue studies tend to be resource intensive due to the need to address spatial and temporal factors which influence pesticide residues in pollen and nectar. Time and resource considerations restrict the number of crops and locations which can be studied. Given these resource constraints, questions remain on how to best optimize the design and number of residue studies for obtaining a robust dataset to refine exposure estimates of bees to pesticides. Factors to be optimized include the number of replicates in each sampling event, the number of sampling events over time, the number of sites per study, and the number of crops to be assessed within and across crop groups. Using available field residue data for neonicotinoids, we conducted an analysis of variability in residue data to address these and other study design elements. For imidacloprid, variation due to analytical measurement was relatively small (coefficient of variation [CV] < 20%). For foliar-applied imidacloprid to citrus and cherry, greater variability among replicate samples is seen with pollen (mean CV =33-64%) vs. nectar (mean CV =24-28%). For blueberry (soil applied), mean residues of imidacloprid in pollen and nectar varied by up to 5X between years for the same sampling period, indicating the importance of capturing inter-annual variability. Across three study sites, imidacloprid residues in blueberry ranged up to an order of magnitude which is likely related to differences in soil texture. For four species of cucurbits, residues in clothianidin varied up to 6X in nectar and 4X in pollen/anthers. The potential influence of the field was explored by comparing the average ratio of maximum to minimum measured concentrations in pollen from 4 crops (potato, tomato, cucumber and pumpkin) grown in common fields from California, Missouri, and North Carolina. Average ratios for potato, cucumber and pumpkin were nearly identical (range: 3X-7X) for each geographic region; however for tomato, the ratio differed by 63X. These results and additional analyses of neonicotinoid residue data will be presented in the context of optimizing field residue study designs for assessing pesticide risks to bees.

Environmental Impacts of Tobacco Products

RP023 A review of carbon monoxide in secondhand smoke of waterpipe

R. Edwards Jr, US FDA / Center for Tobacco Products

Waterpipes (WPs) have been used to smoke tobacco by indigenous peoples of Africa and Asia for at least four centuries.1 A typical WP includes a head where charcoal and tobacco are placed, a body through which vapors pass, a bowl that contains water through which vapors pass, and a hose that leads to the smokers' mouthpiece. WP's secondhand smoke (SHS) is comprised of side stream smoke (SSS) emitted from the WP and exhaled mainstream smoke (EMSS) from the user. Carbon monoxide is a component of the SHS. The burning of charcoal to heat the tobacco for the WP produces the majority of the SHS CO. The reviewed literature shows that CO levels can reach concentrations harmful to individuals exposed to WP SHS.

RP024 Assessing toxicity of tobacco product waste in leachate using chemical analysis and in vitro cell-based bioassays

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Discarded smoked cigarettes are the most prevalent types of litter found in ocean beaches and inland waterways. A discarded smoked cigarette is composed of a filter and unburned matter. It can contain many harmful compounds from the complex mixtures of chemicals in tobacco and tobacco smoke. We hypothesized that discarded smoked cigarettes would leach harmful chemicals into the aquatic environment. To test this, we analyzed the chemical constituents in freshwater and seawater leachate, and assessed the leachates for in vitro biological activity. Chemical identification was conducted by non-targeted analysis using comprehensive two-dimensional gas chromatography coupled to time-of-flight mass spectrometry (GC×GC/TOF-MS). In total, 687 unique compounds were found in both leachates. In the freshwater leachate, 53 were unique to that leachate, and in the saltwater leachate 173 were identified. The in vitro estrogenic receptor (ER), aryl hydrocarbon receptor (AhR) and p53 responses to leachates were further assessed by using the established LUMI-CELLTM ER (BG1Luc4E2), AhR (GeneBLAzer CYP1A1-bla LS-180) and p53 (p53RE-bla HCT-116) cell-based bioassays. Treatments of 10 cigarettes (cig)/L seawater and freshwater leachates showed no significant ER, AhR or p53 activities, while 100 cig/L seawater leachates had significantly higher AhR (2.7 ng/L aryl hydrocarbon toxicity equivalent (TEQ)) and p53 responses (41.9 µg/L mitomycin equivalent (MCQ)) than controls (< 0.17 pg/L TEQ, < 1.4 µg/L MCQ). Cytotoxicity measured by the MTT assay was also higher when cells were exposed to the seawater leachate (relative cell viability < 80% in general). Overall, these results indicate saltwater leachates of tobacco products were higher than freshwater leachates. Ongoing studies are underway to identify specific compounds responsible for the bioactivity.

RP025 Chemical Emissions from Cigarette Butts: A Literature Review

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Globally, 5.6 trillion cigarette butts are deposited into the environment per year, which are some of the most common forms of litter found on beaches and near streams, night clubs, bus stops, roads and streets. Cigarette butts are made of non-biodegradable cellulose acetate, which is used for reducing inhalation of harmful chemicals (e.g., tar and nicotine) during smoking. Many governments have made regulations to sanction penalties for littering of cigarette butts due to the substantial costs to clean them up. However, little is known about the emission characteristics of littered cigarette butts, even though some toxicological studies have shown the association between exposure to cigarette butts and adverse health effects (e.g., shorter life spans for mosquitoes, genotoxicity for house finches, mortality and behavioral modifications for snails). A literature review was conducted in order to identify data gaps related to chemical emissions of littered cigarette butts. We searched articles published prior to November of 2015 on ISI web of science database for the keywords – "cigarette butt" or "cigarette filter" or "cigarette tip" or "tobacco filter". The literature search identified 2382 articles. Only 200 of those articles were deemed relevant to emission characteristics of cigarette butts in general and reviewed in detail for this paper. The review focused on emission from cigarette butts into water and air, and composition of chemicals in cigarette butts. Generally, few studies have been conducted on chemical emissions from cigarette butts. Among those, present studies mainly focus on chemical emissions into water. Only 1 paper published in 1985 investigated airborne emission from cigarette butts. Chemicals detected from extraction studies and water emission experiments include nicotine, amines, pyrroles, PAHs, insecticides, radionuclides, heavy metals and terpenoids. Given the fact that many chemicals present in cigarette butts are harmful, even carcinogenic, more research on emission from cigarette butts is needed, especially airborne chemical emission from cigarette butts which may have different emission characteristics from the water leachate. Some recommendations are given for future work on airborne emission from cigarette butts.

RP026 Considerations of Environmental Effects of Tobacco Products

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On June 22, 2009, the President signed the Family Smoking Prevention and Tobacco Control Act (the Act) into law. The law grants the US Food and Drug Administration (FDA) new authority to regulate manufacture, marketing, and distribution of tobacco products to protect the public health and to reduce tobacco use by minors. On June 25, 2013, FDA announced the first decisions authorizing new tobacco products for introduction into interstate commerce for commercial distribution in the U.S. through the substantial equivalence pathway. FDA considered the environmental effects due to the actions, the authorizing of the products. FDA found no significant impact as a result of the actions. This poster presents the salient considerations in the environmental assessments prepared by FDA for these actions.

RP027 Data Gaps in the Environmental Risk Assessment Process for Tobacco Products

G. Gagliano, US FDA / CTP Office of Science; M. Niazi, US FDA / Center for Tobacco Products

Environmental risk assessment traditionally integrates exposure and effects data to predict potential adverse impacts. Although the high level considerations of environmental risk assessment are well-established, the environmental aspects of tobacco products reach beyond impacts to biodiversity (flora and fauna). The other environmental impacts, which include climate change, land use, water use, eutrophication and acidification, need to be considered as well. As with any emerging issue, all of the information necessary for a risk assessment is not readily available. The data gaps in the environmental risk assessment process for tobacco products are discussed here.

RP028 Sustainability Issues Regarding Smokeless Tobacco Product Manufacture using Findings from Semi-systematic Review on Sustainability of Consumer Goods

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Sustainability entails "maintaining or improving the integrity of the life supporting systems of the Earth"[1] under which humans and nature can co-exist in productive harmony, permitting social and economic provisions for present and future generations. Under Executive Order 13514, Federal agencies are required to develop, implement and annually update a plan that prioritizes actions based on a positive return on investment for the American taxpayer and to meet greenhouse gas (GHG) emissions, energy, water, and waste reduction targets[2]. CTP's mission is to reduce the death and disease from tobacco use. Smokeless tobacco products, such as snuff and chewing tobacco, adversely impact human health. The Agency does not anticipate the use of smokeless tobacco products to decline, based on an evaluation of the Alcohol and Tobacco Tax and Trade Bureau's historical reports. As a result, reduction in energy and water use, as well as GHG and waste generation, are not anticipated. CTP is responsible for protecting human health and safeguarding the human and natural environment. CTP does this by evaluating environmental sustainability related to tobacco product manufacture. Literature research into the sustainability issues related to smokeless tobacco products has identified insufficient information to fully understand sustainability in the products. Therefore, a rapid, systematized research review to explore environmental sustainability related to consumer goods can serve as a surrogate for the understanding of environmental sustainability issues related to smokeless tobacco products to encourage conservation of air, water, and land resources. This approach can also potentially serve as a model for the understanding of environmental sustainability in the context of manufacturing other tobacco products. Various indicators and metrics that help to identify potential problem areas will be explored. [1] Holden, J.P., Daily, G.C. and Ehrlich, P.R. 1995. The meaning of sustainability: biogeophysical aspects. In: Munasingha, M., Shearer, W. (Eds.), Defining and Measuring Sustainability, The World Bank, Washington, D.C. [2] Office of the Press Secretary, The White House. Executive Order 13514: Federal Leadership in Environmental, Energy and Economic Performance. Issued Oct. 5, 2009. Available at: https://www.whitehouse.gov/assets/documents/2009fedleader_eo_rel.pdf. Accessed June 2, 2016.

RP029 Using GIS to Enrich Our Understanding of Tobacco Life Cycle

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Geographic information system (GIS) is a widely used and valuable mapping tool that can depict geographic information in visually stimulating ways. We can use this tool to enhance our knowledge regarding tobacco life cycle – where it is grown, where it is manufactured, where it is consumed, and where it is disposed. We can continue to use this beneficial tool to further learn about the significant environmental impacts of all stages of the life cycle of tobacco, and in turn, we can answer key questions about tobacco and identify topics which may benefit from additional research.

Ecological Effect Models for Assessing the Risks of Pesticides: Ongoing Developments in the US and EU

RP030 Exposure specific species sensitivity – a toxicokinetic-toxicodynamic approach

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Currently in higher tier environmental risk assessment of chemicals, species sensitivity distributions (SSD) are used to describe and to quantify differences in sensitivity among species. When available for multiple species, no-observed-effect-concentrations (NOECs) or ECx, (representing x% of an affected population) usually derived from acute and chronic toxicity tests under constant exposure, are being used to calculate SSDs. In turn, these SSDs are used to extract 'safe' community effect thresholds in the form of an HC5, i.e. the concentration at which 5% of the species are affected. In current ERA practice, the ratio of effect measures and exposure, such as the maximum predicted exposure concentration (PECmax) or the time weighted average (TWA), are being employed to characterize risk ignoring that, depending on environmental conditions, the exposure concentrations are highly variable in space and time. Mechanistic models, such as the General Unified Treshold model of Survival (GUTS), take the process leading to an effect into account and can explicitly cope with time variable exposure. We present the outcome of a GUTS workshop held in March 2015 where species sensitivity distributions under time variable exposure have been studied. Our results point towards an unrecognized problem with the widely used SSDs and HC5 values: they could depend on the exposure pattern and differ a lot for exposures with the same time weighted average concentration but different temporal profiles. By using GUTS to analyze and compare species sensitivities, we can overcome the dependence of time and exposure patterns, and extrapolate toxicity to other exposure scenarios. Furthermore, we can develop a more thorough understanding of the underlying toxicity mechanisms by quantifying organism internal toxicity thresholds in conjunction with compensating processes.

RP031 Modelling survival: exposure pattern, species sensitivity and uncertainty

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Stressor effects on survival over time are studied in a wide range of disciplines. A comprehensive survival data analysis entails specifying where the stressor is quantified, how to model compensatory processes and whether death is best viewed as a stochastic or a deterministic event. The General Unified Threshold model of Survival (GUTS) provides a framework for deriving consistent model equations for these different choices and assumptions. On this poster, we would like to present the results of a GUTS workshop held in March 2015. During the workshop and in the after math, we tested the ability of GUTS to predict survival of aquatic organisms across different chemical exposure patterns, time scales and species. We found that prediction uncertainty can be reduced by increasing the number of individuals in the calibration experiments. We here propose a method to optimize experimental designs using synthetic data. Our results further demonstrate that different temporal patterns of chemical exposure, even with the same time weighted average (TWA) concentrations, may result in different species sensitivity rankings as well as different estimates for safe concentrations. We conclude that the interplay of exposure pattern and species sensitivity deserves more attention and systematic investigation. The GUTS framework offers a straightforward and consistent platform for both scientific and regulatory questions on the dynamics of survival under stress conditions. This poster summarizes the results of the three working groups with different focus.

RP032 Intake and body burden: Complementary approaches in characterizing risk to wildlife associated with pesticide use

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In assessing risk from pesticides to wildlife, exposure and effects metrics take a variety of forms. Exposure estimates may include dietary concentrations, total daily intake (TDI), body burden and application rates. Effects metrics are typically derived from standard tests, including acute oral gavage, and subacute and chronic dietary studies, in units of mg/kg bw, and mg/kg diet or mg/kg bw/d, respectively. For mortality risk, effects metrics are typically compared to: (1) body burden, or (2) TDI. The latter is far more common in regulatory risk assessment of pesticides, whereas the former has most recently been applied in the USEPA's pilot biological evaluations for threatened and endangered species. These two approaches can give strikingly different results for the same pesticide use and receptor. Differences are due in part to the inherent assumptions associated with the two methods. When body burden estimates are used to estimate mortality risk, it is implicitly assumed that effects will be equivalent to those estimated with oral gavage exposure for the same body burden. However, distribution over time within the organism will determine the amount of active ingredient at the site(s) of toxic action, and thus an equivalent body burden does not necessarily equate to equivalent toxic effects. Conversely, when TDI estimates are employed, mortality estimates are limited by the exposure duration of the test (for birds, typically five days). Depending on the pesticide, the receptor, available fate and toxicity data, and assessment tier, one approach may be more appropriate than another. Body burden estimates require knowledge of the rates at which the active ingredient is metabolized to non-toxic degradates and/or eliminated. When pesticides degrade rapidly in the environment and are quickly metabolized and/or eliminated, risk estimates based on peak exposure may be similar for the two approaches, or TDI may be more conservative. However, if the pesticide is somewhat persistent, the body burden approach may result in far more conservative risk estimates than the TDI approach. The TDI and body burden approaches can be used together to enable a comprehensive quantitative risk characterization that overcomes some of the uncertainties in both methods. Accordingly, a tandem approach may lead to more informed risk management. Case study examples will be provided.

RP033 Determining the probability of pesticide exposure for birds during migration

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Many species of birds that spend their summers in North America migrate to and from their wintering grounds in the southern United States and in central and South America. Migration occurs during late summer/early fall and during the spring. Most likely, species have evolved so that timing of migration coincides with availability of food sources. In the spring, emerging insects provide the calories and energy requirements necessary for birds to stop and fuel up between migration flights. If the migration period coincides with insecticide applications and birds stop in agricultural fields or adjacent areas (that receive spray drift), they may be exposed to pesticides through consumption of contaminated food items (e.g., insects), and/or contact with treated foliage or drinking water. The number or proportion of birds within a population that may be exposed to a pesticide is an important consideration for translating potential effects from individuals to understand population level consequences. In this approach, the probability associated with different magnitudes of exposed individual birds of a population is estimated. The following factors were considered: the migration range, percent of the range representing potential pesticide use sites, proportion of use sites that are treated, number of stops made during migration, and likely habitats used by the bird species when stopping during migration. An example is presented to demonstrate the likelihood of exposure of the migrating Kirtland's warbler (Setophaga kirtlandii) to the organophosphate insecticide, diazinon.

RP034 Population modelling and algae flow-through toxicity tests as higher-tier option in pesticide risk assessment – results for different compounds

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Algae flow-through toxicity tests can be a useful higher-tier option to support the aquatic risk assessment of pesticides. A flow-through setup enables the evaluation of complex, time-variable exposure profiles. This allows for example the assessment of population-level effects after multiple pulsed pesticide exposure to algae. It is possible to observe the inhibition of algal growth directly as visible loss of population biomass on the one hand, and subsequent population recovery to an initial steady-state level on the other hand. A combination of the test system with an algae population model significantly enhances the interpretation of the experimental data. A priori, the test conditions and expected results of the flow-through test can be simulated. If the simulated outcome indicates that a flow-through test would be useful, the model can be used to design the test according to specific needs of a given risk assessment and to maximize the information that is gained from the experiment. The application of a generic exposure pattern in the flow-through test and simulation of the test results validates the model for further predictions of population-level effects of the specific test chemical and algae species. As a consequence the validated model can be used for extrapolations to any other exposure regime. This approach has been tested with different algistatic toxicants and two standard algae test species. Different types of pulsed exposure events, including a range of repeated peaks as well as more constant exposure periods were tested. The environmental conditions during a test, the physiological properties of the algae and toxicity data obtained from standard growth inhibition studies were the only necessary model inputs for simulation of flow-through tests. Experimental and modelling results for different compounds will be presented and an application of this approach within a regulatory context will be discussed.

RP035 Multi-phase distribution characteristics and risk assessments of pesticides in aquatic environments from an urban-industrial impacted coastal bay

Z. Wang, Key Laboratory of Urban Environment and Health; R. Li, Inst of Urban Environment, Chinese Academy of Sciences; C. Yan, Insitute of Urban Environment, Chinese Academy of Sciences

China is one of the largest producer and consumers of pesticides in the world today. Along with the widespread use of pesticides and the development of urbanization and industrialization, there is a growing concern for quality in aquatic ecosystems. To discover the distribution characteristics and complex risk in an urban-industrial impacted coastal bay, a multi-residue analysis method based on gas chromatography with electron capture detection (GC-ECD) was developed, and the residues of commonly used pesticides in China including organochlorine, organophosphorus, carbamates, pyrethroids, azoles, amides and anilines were analyzed in seawater, sediments and biota collected from various sites. Mean recoveries were satisfactory with 72-103%. A total number of 86 pesticides were detected and the occurrence and the risk to human and fish were then assessed. Most of pesticides with high frequency were medium or low toxicity except for DDTs. DDTs were the significant contaminant and the widely used dicofol was the new sources. The ratios between the parent DDT compound and its metabolites [(p,p'-DDE+p,p'-DDD)/p,p'-DDT] was determined as a useful indicator to provide information on the pollution source and indicate new inputs of the technical DDTs. The low ratio of (DDE+DDD)/DDT (< 1.0) is indicative for fresh DDTs application and a ratio much greater than 1.0 is indicative aged (microbial degraded) DDTs. In addition, the ratio of o,p'/-DDT/p,p'-DDT was also obtained to distinguish whether the DDT pollution caused by technical DDT or by dicofol. The spatial variation of pesticide distribution was linked with the fishing ships, where antifouling paint containing DDT was still utilized as an active component. Health risks from seawater and clams were assessed, respectively, where clam consumption was medium while pesticide in water posed great risk to fish. DDTs, dicofol, triazophos, fenvalerate, butachlor amd terbufos have become the significant pesticides in this investigation, indicating that these pesticides should be considered in aquatic ecosystem risk management and measures should be taken to decrease pesticide residues in order to improve the quality around urban-industrial regions. (The authors acknowledge financial support by the open fund from the State Key Laboratory of Environmental Criteria and Risk Assessment in Chinese Research Academy of Environmental Sciences (SKLECRA2013OFP13), and the Natural Science Foundation from Ningbo City (201301A6107013)).

RP036 Toxicity of various insecticides used for management of Asian citrus psyllid in Florida citrus against honeybees under laboratory conditions

X. Chen, Univ of Florida; L. Stelinski, Univ of Florida / Entomology and Nematology

The Asian citrus psyllid, Diaphorina citri Kuwayama is a major pest of citrus crops worldwide. A wide range of insecticide has been used to manage D. citri in Florida. However, in areas shared by citrus growers and beekeepers such as Florida, the use of insecticides can increase the risks of honeybee death and honey contamination. The objective of this investigation was to determine the toxicity and hazard of four different insecticide mode of action: neonicotinoid (imidacloprid), pyrethroid (fenpropathrin), organophosphate (dimethoate), and spinosyn (spinetoram) on honeybee workers (Apis mellifera L.). These insecticides are heavily used on Florida citrus. For this experiment, five to seven doses of each insecticide were tested, each one in triplicate. Each replicate was conducted on a group of 5 honeybees; all insecticide dilutions were made in acetone. For the control treatment, a similar procedure was performed, also in triplicate, but in which acetone alone. An adult mortality was assessed 48 h after exposure to treatments. Resulting LD_{50} values of test insecticides ranged from 0.85 to 0.13 mg/L. Hazard quotients ranged from 16.35 to 81.82. Also, chronic toxicity was investigated for imidacloprid, fenpropathrin, dimethoate and spinetoram at $LD_{0,}$ LD_{10}, LD_{25}, LD_{50}, LD_{75} and LD_{95} doses 24, 48, 72, 96, 120 and 144 hour after treatment. Results indicated that imidacloprid exhibited extreme chronic toxicity to adult A. mellifera. Fenpropathrin, dimethoate and spinetoram caused moderate and low mortality rates of A. mellifera with directly application at lethal and sublethal dosages of application for D. citri.

Epigenetics and Environmental Exposures: Mechanisms and Effects from Invertebrates to Fishes

RP037 Multi-stressor impacts on the development and maintenance of epigenetic regulation in larval and adult zebrafish (Danio rerio)

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As the human population increases, increased anthropogenic stress is placed on the environment. Venlafaxine is an antidepressant found downstream of wastewater treatment plants, at 1 µg/L. Also, increased water temperature and decreased oxygen content have been observed as a result of human activity. These effects are being studied in a multi-stressor approach to elucidate the combinatory effects of multiple chronic stressors. Specifically, the effects of venlafaxine, increased temperature and decreased oxygen on microRNA (miRNA) levels in larval and adult tissue were studied to determine sub-lethal, epigenetic effects on zebrafish. Adult zebrafish were chronically exposed to treatments for 21 days. Cardiac, liver, gonad, and muscle tissues were removed, RNA was extracted and RT-qPCR was performed on specific miRNA known to be related to genes involved in hypoxia, heat stress and response to contaminants to determine their relative abundances. Further, zebrafish embryos were exposed from 0 hpf to 96 hpf with an examination of the same miRNA and genes as in adults. Effects on juveniles and adults will be discussed. Understanding the epigenetic impact of multiple environmental stressors on different life stages and across generations will help us to understand the full impact on the aquatic ecosystem. Due to the conserved nature of miRNA, this will improve our understanding of the effects that environmental stressors have on epigenetic regulation.

RP038 Toxicity effects of PCB- 95 enantiomers on early neurodevelopment of zebra fish larvae

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Early neurodevelopment is influenced by numerous genetic and epigenetic factors, with long-lasting effects on brain function and behavior, but the interface of these factors interactions are poorly understood. PCB-95 is a chiral molecule identified as one of the most potent neurotoxic PCB congener. Enantiomers of chiral PCBs have different neurotoxic effects as individual stereoisomers can interact differentially with other chiral molecules, such as enzymes and biological receptors. We are investigating the effects of PCB-95 enantiomers on neural development utilizing high-throughput analyses of behaviors in zebra fish larvae. Zebra fish (Danio rerio) larvae at 7 days post fertilization (7dpf) are exposed to racemic and individual enantiomers of PCB-95 concentrations (0.25, 0.5, 1, 5 ppm). Exposure time is 1 day with 3 day incubation period. Each treatment consists of four replicates with two controls (egg water and dimethyl sulfoxide). Morphological deformations such as pericardial and yolk sac edemas, curved body and stunted growth are assessed and those larvae are excluded from the behavioral assays. The performance of developing zebra fish in habituation assays is tested by exposing zebra fish larvae to a visual pattern presented beneath the well plate (a red 'bouncing' disk which runs in a straight line at the rate of 1.50 cm/s at the upper half of the well plate). Images are captured every 6 s for 150 minutes. Swimming behavior around the well is quantified based on either the animal's position or its turning behavior. PCB levels within exposed larvae are analyzed using GC/ECD. Data are analyzed using PROC GLM in SAS software. Preliminary data show morphological deformations with the embryonic racemic PCB-95 exposure. We expect to observe increased mortality rates and in survivors altered response to the visual startle stimulus such as mean percent avoidance, mean swim speed and mean percent still. Increased levels of morphological and behavioral deficits may be expected with increased exposure. Each PCB-95 enantiomer can have different potencies, and these may differ from that of the racemic mixture in neurodevelopment of zebra fish larvae. Outcomes from our study will reveal windows of susceptibility and underlying mechanisms of prenatal exposure of PCB- 95 with relevance to ecological consequences and human exposure.

RP039 Impacts on genome-wide DNA methylation pattern in early-life stages zebrafish (Danio rerio) after triclosan exposure

E. Falisse, Univ of Namur; A. Chatterjee, I. Morison, Univ of Otago; F. Silvestre, Université de Namur ASBL / Dept of Biology

DNA methylation is a dynamic epigenetic mark that contributes to gene regulation and genome maintenance. It appears mostly on cytosine residues within a CpG context. There are increasing evidences that toxicant exposure can alter methylation profiles, particularly during embryogenesis when DNA methylation patterns are established. In some extent these modifications can possibly last in adulthood and might be inherited through successive generations. In the present study, we analysed the effects of triclosan (TCS) at 50 and 100µg/L on DNA methylation during early zebrafish (Danio rerio) embryogenesis, during 7dpf. We used Reduced Representative Bisulfite Sequencing (RRBS) to profile genome-wide methylation pattern at single-nucleotide resolution. Using DMAP pipeline we have identified significantly differentially methylated fragments (DMFs) between conditions (cut-off q-value of < 0.01 after FDR adjustment and with percent methylation difference ≥15%). A total of 171 DMFs were identified, mostly between fish exposed to 50µg/L and 100µg/L (58% of DMFs). The main biological pathways involved were the metabolic, the cellular, the biological regulation and the developmental processes. Almost 50% of the DMFs resided within CpG island shore (defined as 2 Kb from either side of a CpG island core) while 48% of the DMFs were outside any CpG feature. These results highlighted the relevance of "non-CpG island" regions during genes regulation after stress exposure. Among all DMFs, we identified 51 fragments overlapping with intronic regions, 18 with exonic regions and only 4 fragments in a gene promoter region. 43 corresponding genes were selected for gene expression analysis using high throughput quantitative PCR (Fluidigm Biomark-HDTM system). These data will provide a deep understanding of the links between gene regulation and the modifications of the methylation landscape at specific CpG island and genomic features. Overall this study emphasizes the epigenetic effects of an exposure to TCS during early embryogenesis and the necessity to take into account the possible long term and transgenerational impacts of pollutant exposure during developmental stages. [1]Chatterjee A, Stockwell, PA, Horsfield, JA, Morison, IM, Nakagawa, S. Base-resolution DNA methylation landscape of zebrafish brain and liver. Genomics Data 2014 [2]Stockwell PA, Chatterjee A, Rodger EJ, Morison IM. DMAP: differential methylation analysis package for RRBS and WGBS data. Bioinformatics 2014

RP040 Does exposure to Bisphenol A or Ethinyl estradiol alter gonad structure and DNA methylation patterns in the aquatic turtle, Chrysemys picta?

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Environmental endocrine disrupting chemicals, such as Bisphenol A (BPA) and ethinyl estradiol (EE2), are widespread in aquatic habitats. Based on our previous work, we expected BPA and EE2 to disrupt sexual differentiation and override temperature dependent sex determination in painted turtles (Chrysemys picta). We incubated farm-raised turtle eggs at the male-producing temperature (26°C) and randomly assigned treatment groups: control, vehicle control, 0.02 µg EE2/egg and 0.05 or 5 µg BPA/egg. Doses were administered by spotting eggs during the temperature sensitive period. Subsequent hatchlings were reared for nine months, and tissues were harvested from euthanized hatchlings. One gonad from each turtle was sectioned and stained for histological evaluation of phenotype. Our previous results have indicated BPA exposure leads to demasculinized testes in hatchlings. The persistence of this condition within the gonad approaching the juvenile stage was evaluated. Finally, differences among treatments with respect to DNA methylation in the CpG promoter regions were evaluated. Collectively, epigenetic and anatomical changes in germ cells may have a significant impact on already struggling turtle populations.

RP041 Epigenetic Biomarkers in Herring Gulls From Contaminated And Reference Sites

E. Boulanger, McGill Univ / Natural Resource Sciences; D. Crump, Environment and Climate Change Canada; J. Head, McGill Univ / Natural Resource Sciences

Early life stages are extremely sensitive to exposure to environmental contaminants. This is also the period when epigenetic marks such as DNA methylation are established. Recent research suggests that disruptions to the epigenome early in life can cause persistent, lifelong consequences in individuals. The goal of this project was to evaluate global DNA methylation as a biomarker for embryonic exposure to environmental contaminants in herring gull (Larus argentatus), a colonial fish-eating bird that is a long-established bioindicator species in the Great Lakes. Research has shown that DNA methylation is affected by many classes of contaminants, (e.g. metals, organic contaminants, pesticides), in many animal models. We hypothesized that herring gulls living and feeding in highly contaminated sites would exhibit DNA hypomethylation compared with individuals from control sites. Fertile unincubated herring gull eggs were collectedfrom 3 different colonies Lake Ontario. Previous research indicates that eggs from these colonies exhibit a gradient of contaminant levels (Hamilton Harbour >Salmon Island >Cornwall). Eggs were artificially incubated until piping, and blood, brain and liver tissues were collected from the embryos. The remaining egg contents were preserved for chemical analysis. Global DNA methylation was assessed in each tissue type via LUminometric Methylation Analysis (LUMA). Levels of global DNA methylation were extremely stable across individuals (for example, average percent DNA methylation ranged from 65.67-70.46% in the blood samples), and there were no significant difference among the 3 herring gull colonies. Significant differences were detected in DNA methylation levels across tissue type (blood>brain>liver), and these were consistent among the herring gull colonies in Cornwall, Hamilton Harbour, and Salmon Island [$p < 0.0001$]. Future analyses will focus on the levels of contaminants in the eggs and methylation specific genes within the samples that were collected.

RP042 Network-inspired analysis of epigenetic responses to environmental stressors in bivalve molluscs

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Bivalve molluscs display a remarkable tolerance to the effects of marine pollution, particularly in the case of Harmful Algal Blooms (HABs) caused by proliferations of toxic dinoflagellates. In addition to the obvious impact for marine ecosystems and aquaculture industries, the accumulation of toxins on bivalve tissues during HABs is also responsible for acute intoxication syndromes in human consumers and genotoxic effects at sublethal concentrations observed in in vitro experiments. Consequently, there is great interest in developing innovative biomarkers for HABs monitoring. The present work builds on the transcriptomic response of the mussel Mytilus to a laboratory-controlled HAB exposure using low concentrations of the dinoflagellate Prorocentrum lima, which produces toxins that causes Diarrhetic Shellfish Poisoning. Our findings reveal sharp changes in gene expression patterns, which are consistent with the consensus responses of oysters to different environmental stressors. In order to identify gene expression patterns specifically linked to a single stressor and those that correspond to a general response to stress, publicly available data series were analyzed using co-expression networks, an approach selected based on its ability to integrate information. The identification of genes showing coordinated expression changes provides a potential source of highly specific biomarkers. More specifically, this approach has been applied to expression data of chromatin-associated genes (i.e., histone variants and histone modifying enzymes) providing relevant insights into the epigenetic response of bivalves to stress by abiotic factors. Future directions involve the validation of specific expression patterns in bivalves exposed to HABs in time-series studies. Overall, this work provides insights into the molecular mechanisms underlying the tolerance of bivalves to environmental stress, laying the foundations for the development of innovative tools for biomonitoring of HABs.

RP043 Crude oil-dispersant mixture induced spermatogenesis defects in Caenorhabditis elegans: implicating risk of tumorigenesis

X. Pan, B. Zhang, East Carolina Univ / Biology

The reproductive health impacts associated with crude oil-dispersant (Oil-dis) mixture exposure was investigated using the model organism Caenorhabditis elegans (C. elegans). Previously we reported that exposure to crude oil and dispersant resulted in reproductive defects such as decreased progeny numbers and increased germ cell apoptosis. In this study, we show that crude oil-dispersant mixture also affected reproduction by inducing abnormal sperm during the process of spermatogenesis. L4 larvae of wild type N2 hermaphrodites were exposed to different dilutionsofoil/dispersant mixture (20:1) for 24h, young adults were dissected and subjected to DAPI staining. Results showed that the abnormal immature sperm were significantly increased in the gonad arms of treated animals compared to controls. We further explored the oil-dispersant mixture toxicity effects on spermatogenesis by using a male C. elegans strain. After 48h exposure to oil- dispersant mixture, spermatids appeared with abnormal morphology including irregular shapes of the spermatid membrane and unexpected tails induced by dispersed oil. Significantly we utilized puf-8; lip-1 tumor sensitive strain to test the cell fate of immature sperm induced by Dis-Oil mixture treatment. Findings suggested increased tumor occurrence in exposure animals. Our study suggests that oil-dispersant mixture induce toxic effects on reproduction by not only affecting oogenesis but also affecting spermatogenesis and pose tumorigenesis risk. Global microRNA profiling and pathway analysis indicate the microRNA regulation of stress response and observed reproductive toxicity.

RP044 Effects of agro-pesticide cypermethrin on haematology of a freshwater catfish Mystus cavasius

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Cypermethrin is widely used as a pesticide in agriculture of Bangladesh. Since most of the agricultural lands of the country are in the floodplains, pesticides are readily leached into immediate aquatic habitat. Gangetic Mystus, Mystus cavasius, is a small freshwater catfish commonly available in the inland freshwater habitats close to agricultural lands. Therefore, a short term definitive exposure experiment was conducted to evaluate changes in hematological parameters of M. cavasius exposed to lower to higher concentrations of the synthetic pyrethroid pesticide cypermethrin. Adult female and male of M. cavasius were exposed to three triplicate concentrations of cypermethrin – 4 µg/L, 8µg/L and 16 µg/L and control (0 µg/L) for a period from Late June to Late August 2015; 40 fishes (20 female and 20 male) each per 200-L cement cistern were reared. Monthly sampling of blood glucose, haemoglobin (Hb), red blood cells (RBC) and white blood cells (WBC) were done. In both female and male, blood glucose levels increased significantly due to toxicity of cypermethrin. Blood glucose level (Mean ±SE) in control female were between 5±0.59 to 5.53±0.53 mmol L^{-1} and in treatment from 6±0.15 to 10.13±0.53 mmol L^{-1} and in male it ranged from 4.9±0.25 to 5.3±0.57 mmol L^{-1} in control and from 5.73±0.14 to 9.6±0.47 mmol L-1 in treatment. Blood Hb levels in both female and male dropped significantly due to exposure to cypermethrin. Hb (Mean ±SE) in control female ranged from 8.6±0.34 to 9.5±0.59 g dL^{-1} whereas it ranged from 4.6±0.31 to 7.86±0.29 g dL^{-1} in treatments. In male it ranged from 9.06±0.43 to 9.8±0.75 g dL^{-1} in control and 4.8±0.41 to 8.2±0.30 g dL^{-1} in treatments. RBC count also dropped significantly as an influence of cypermenthrine exposure. In female, monthly RBC levels (×106 mm-3; Mean ±SE) ranged from 0.59± 0.06 to 1.88± 0.95 in treatments compared to 2.03±0.36 to 3.27±0.03 in control. In male, the value ranged from 2.48±0.014 to 2.61±0.06 in control and between 0.38±0.03 to 1.88±0.36 in male. WBC counts elevated in most of the female and male fishes exposed to different concentrations of cypermethrin. These results indicate that hematological parameters may be useful as a diagnostic test for cypermethrin exposure in aquatic animals and calls for the limited and cautious use of cypermethrin.

Radionuclides in the Environment, Including Accumulation in Biota

RP045 Effect of Migratory Life History on North Pacific Albacore (Thunnus alalunga) Uptake of Radiocesium

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The Fukushima Dai-ichi power station released radionuclides into the Pacific Ocean off eastern Japan in 2011. Previous radionuclide releases to the ocean were either of much lower magnitude, released over a much larger area or released in a continuous format. This pulsed-release provided an opportunity to examine how differences in migration routes and population structure of North Pacific albacore affected their uptake of radiocesium. Albacore samples collected (2011-2015) from two regions (North and South of 40° N) in the eastern Pacific were tested for ^{134}Cs and ^{137}Cs. Pre-disaster control samples (2008) were also tested. Measurements thus far have shown almost no ^{134}Cs in Southern region albacore and a mix of presence/absence of ^{134}Cs and elevated ^{137}Csin Northern region albacore. North albacore showed a positive correlation of ^{134}Cs and elevated ^{137}Cs with length, which indicated that the smaller individuals generally had not recently migrated from waters with ^{134}Cs such as eastern Japan or the North Pacific Transition Zone. The spatially variable concentrations of ^{134}Cs suggest latitudinal migratory differences and limited mixing of N/S albacore in the eastern Pacific. Albacore tissue contained radionuclide levels orders of magnitude less than both US FDA food standards and natural ^{40}K concentrations.

Recovery of Pelagic Fishery Populations Following Oil Exposures

RP046 Combined effects of oil exposure, temperature and UV-radiation on buoyancy and oxygen consumption of embryonic mahi-mahi, Coryphaena hippurus

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The timing and location of the 2010 Deepwater Horizon (DWH) incident within the Gulf of Mexico resulted in crude oil exposure of many commercially and ecologically important fish species during the sensitive early life stages. Gulf of Mexico pelagic species, such as mahi-mahi (Coryphaena hippurus), typically produce small and rapidly developing buoyant embryos. These pelagic embryos and larvae that float in the upper layers of the water column are likely directly exposed to PAHs in surface oil slicks, along with multiple concurrent stressors, such as UV-radiation and high temperature. Mahi-mahi embryos typically become negatively buoyant a few hours before hatch, when raised in ambient, non-stressful conditions. Previous data from our lab has shown that negative buoyancy in mahi-mahi embryos correlates to a drastic increase in oxygen consumption. This study examined the effects of multiple simultaneous stressors on buoyancy change and oxygen consumption. When combined with oil-exposure, stressors such as temperature and UV-radiation induce negative buoyancy at earlier stages of development, correlating to significant increases in oxygen consumption for extended periods before hatch. Alterations to the timing of negative buoyancy onset and increased oxygen consumption likely have detrimental ecological implications for these developing embryos due to increased energy depletion and potential sinking to unfavorable hatching depths. This research was made possible by a grant from The Gulf of Mexico Research Initiative. Data are publicly available through the Gulf of Mexico Research Initiative Information & Data Cooperative (GRIIDC) at https://data.gulfresearchinitiative.org.

RP047 Gene expression analysis of the impacts of crude oil toxicity on nitrogenous waste excretion in mahi-mahi (Coryphaena hippurus) early life stages

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The timing and location of the 2010 Deepwater Horizon (DWH) incident within the Gulf of Mexico coincided with the spawning of many commercially and ecologically important fish species, such as mahi-mahi (Coryphaena hippurus). Recent studies by our lab have shown that oil exposure increases oxygen consumption of developing mahi-mahi larvae and that this increased metabolic demand is likely fueled by increased protein catabolism, as evidenced by increased nitrogenous waste excretion. Additionally, a recent RNA-seq study by our lab and others revealed a number of oil-responsive genes in early life stage mahi-mahi associated with nitrogenous waste excretion. Similar to other teleosts, mahi-mahi avoid the toxic build-up of ammonia by being ureotelic during the embryonic stage and gradually switch to being ammoniotelic around hatch. Thus, any disruption in the timing of these processes could indicate significant physiological impacts with implications for survival. In this study, we followed the mRNA expression changes for four genes involved in nitrogenous waste excretion over the initial 96 h of life: Rhag and Rhbg (ammonia transporters), SLC14a2 (urea transporter) and NHE3 a Na^+/H^+exchanger potentially involved in ammonia transport. Initial studies using control fish revealed the expected expression profiles for 3 of the 4 genes based on their putative roles. Rhag and Rhbg both showed progressive increases in expression over time, starting at the time of increased ammonia excretion and peaking during the larval stage at 96 h. SLC14a2 peaked before hatch at 30 hpf, at a time where urea excretion reached peak levels, and steadily decreased thereafter. However, NHE3 revealed no significant changes over time. Current efforts are focusing on examining oil-induced responses to these gene expression profiles and utilizing in situ hybridization to localize the tissue-specific expression of these genes. This research was made possible by a grant from The Gulf of Mexico Research Initiative. Data are publicly available through the Gulf of Mexico Research Initiative Information & Data Cooperative (GRIIDC) at https://data.gulfresearchinitiative.org

RP048 Foraging behavior and olfactory capacity of Mahi Mahi (Coryphaena hippurus) exposed to crude oil from the Deep Water Horizon event

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During the Deep Water Horizon event in 2010 approximately 4 million barrels of crude oil were spilled into the northern Gulf of Mexico overlapping with the spawning habitat of many ecologically and commercially important species of pelagic fishes, including Mahi Mahi (Coryphaena hippurus). Mahi Mahi are one of the top predators in the pelagic ecosystem and rely on olfaction and vision to find high quality prey patches in a pelagic food desert. In fishes, the olfactory epithelium is in direct contact with the aquatic environment making it particularly vulnerable to contaminants, such as crude oil, in the water. This study assesses the olfactory performance and behavioral response to prey cues in juvenile Mahi Mahi. Previous studies using a flume choice system have suggested that unexposed juvenile Mahi Mahi have the ability to detect and avoid crude oil, whereas, oil exposed (18 ug/L for 24-hours) conspecifics do not, and instead show attraction to crude oil at high concentration. Following these results, we measured the electrophysiological response of control and oil exposed juvenile Mahi Mahi to crude oil and prey cues using an electro-olfactogram (EOG) and measured the behavioral response to olfactory prey cues using video analysis with Loligo Systems Lolitrack software. We expect EOG data to show a reduction in olfactory performance with increasing concentration of crude oil. Altered physiological and behavioral patterns following oil exposure could result in inadequate foraging and reduced survival in this ecologically and commercially important species.

RP049 The effects of crude oil co-exposed with multiple stressors on the metabolomic profile of mahi mahi (Coryphaena hippurus) embryos

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The timing of the Deepwater Horizon oil spill in 2010 overlapped with the spawning of several ecologically and economically important fish species, including the mahi mahi (Coryphaena hippurus). Several studies have shown that PAHs released into the marine environment from the DWH spill caused significant photo-enhanced toxicity to aquatic organisms. Mahi mahi eggs are transparent and positively buoyant until right before hatch (40 hpf). Their position within the water column during development makes them at risk for photo-enhanced toxicity with additional stressors such as temperature and salinity fluctuations. In this study, mahi mahi (6 hpf) were exposed to OFS crude oil for 24 h. During the 24 h oil exposure, mahi mahi were co-exposed to UV light. After the UV exposure, embryo respiration and the rate of buoyancy change was quantified. The embryos exposed to UV became negatively buoyant earlier in development than the non-UV exposed embryos, correlating with a significant increase in oxygen consumption in the UV exposed embryos. The effects on buoyancy change and oxygen consumption were further amplified when the same experiment was performed at increased temperature (30°C) or decreased salinity (30 ppt). A subset of embryos from each experiment was used for metabolomics analysis using GC/MS. The FIEHN metabolomics library was utilized to elucidate potential metabolic pathways involved with changes in buoyancy and oxygen consumption in mahi mahi exposed to multiple stressors. This research was made possible by a grant from The Gulf of Mexico Research Initiative. Data are publicly available through the Gulf of Mexico Research Initiative Information & Data Cooperative (GRIIDC) at https://data.gulfresearchinitiative.org.

RP050 Effects of oil exposure on vision in a pelagic marine predator, mahi-mahi

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During the Deepwater Horizon spill millions of barrels of oil were released into the Gulf of Mexico at peak spawning periods of commercially important fishes, including the mahi-mahi (Coryphaena hippurus). Developmental abnormalities have been shown to result from polycyclic aromatic hydrocarbons (PAHs) present in the oil, and could impact the vision of these fish. In this study, larval mahi-mahi, ranging from 1 day post hatch (dph) to 11dph, were assessed for visual function using the flicker fusion principle to monitor an optomotor response. Embryonic and juvenile mahi were exposed to high energy water accommodated fractions (HEWAFs) of weathered oil and optomotor response was determined. Visual acuity in juvenile mahi was significantly reduced following exposure to oil. Fish were taken from each dph stage and the expression of several genes associated with vision were measured using qPCR along with histological sections of the eye taken. The results of this study provide information on visual and behavioral impacts of oil on fish vision. This research was made possible by a grant from The Gulf of Mexico Research Initiative. Data are publicly available through the Gulf of Mexico Research Initiative Information & Data Cooperative (GRIIDC) at https://data.gulfresearchinitiative.org.

RP051 Molecular and anatomical links between crude oil cardiotoxicity and osmoregulatory function in pelagic fish larvae

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Crude oil has been shown to affect a broad range of teleost species from a variety of habitats through impacts on cardiac function and morphogenesis. Polycyclic aromatic hydrocarbons (PAHs) from crude oil and other sources (e.g., urban runoff) are cardiotoxic to the developing fish heart, acting through multiple mechanisms, from disruption of ion channel function to activation of the aryl hydrocarbon receptor. In recent decades, the presence of edema in fish embryos and larvae has become a standard bioindicator of cardiotoxicity. However, the different ways that edema forms in fish from different habitats (i.e., freshwater vs. marine, pelagic vs. demersal) has not been rigorously examined. In particular, the accumulation of edema in marine fish embryos is paradoxical, given that they are hyposmotic to the surrounding environment. Our studies on several species of fish with pelagic yolk sac larvae provide novel insight into mechanisms of edema formation in marine fish. Pelagic marine larvae are characterized by large marginal finfolds encompassing a voluminous subdermal space. A relationship between this unique morphology was first related to osmoregulation and buoyancy control by Shelbourne over sixty years ago, but there has been little progress in the decades since, particularly at a molecular scale. Using evidence obtained from nine freshwater and marine fish species exposed to crude oils from different parts of the world, we show how patterns of cardiogenic edema are shaped by species-specific differences in developmental anatomy and ionoregulatory physiology. RNASeq studies in pelagic Atlantic haddock embryos and larvae provide insight into molecular pathways involved in edema formation, altered ion and fluid balance and lymphangiogensis. These data suggest there may be poorly understood adverse outcomes pathways related to osmotic gradients and water movement within embryos, the latter causing extreme shifts in tissue osmolality.

RP052 Developmental toxicity of 2- and 6-hydroxychrysene in zebrafish embryos

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Oil spills are one of the primary sources of polycyclic aromatic hydrocarbons (PAHs) in marine environments and are subject to biotic and abiotic weathering, leading to alterations in physical and chemical characteristics of PAHs. PAHs can undergo photochemical oxidation, forming oxygenated photoproducts that have the potential cause adverse ecological effects. Among the PAHs found in crude oil, chrysene is one of the most persistent within the water column and is susceptible to photo-oxidation, resulting in production of oxygenated derivatives such as 2- and 6-hydroxychrysene. However, very little is known about the toxicity of hydroxylated PAHs. Previously, we showed that exposure to 2- and 6-hydroxychrysene can adversely affect the development of zebrafish embryos. Therefore, the goal of this study was to identify a sensitive window of embryonic development for 2- and 6-hydroxychrysene-induced toxicity. Embryos were statically exposed starting from 2, 5, 10 or 24 hours post-fertilization (hpf) to 0.5 and 5 μM of 2- or 6-hydroxychrysene for 74hrs. At 76 hpf, there was a significant decrease in survival after initiation of treatment with 5 μM 6-hydroxychrysene at 2 hpf, whereas no difference in percent survival (relative to vehicle controls) was observed following initiation of 2-hydroxychrysene treatment at any of the developmental stages tested. However, the prevalence of cardiac deformities was significantly higher after treatment with 2- and 6-hydroxychrysene compared to vehicle controls. These findings suggest that there is a critical time during early development when embryos show heightened sensitivity, leading to decreased survival after exposure to specific PAHs. Moreover, relative to vehicle controls, there was an increase in the intensity of yolk sac-localized fluorescence after treatment with either 2- or 6-hydroxychrysene. However, 2-hydroxychrysene resulted in the highest fluorescence intensity, suggesting differential uptake and/or metabolism between these two compounds. Overall, these studies highlight the regioselective impacts of hydroxylated PAHs on cardiac development and survival of fish embryos. Our findings raise the need to identify mechanisms involved in the toxicity of these compounds to assess the potential risks of oil spills on fish populations. This research was made possible in part by a grant from BP/The Gulf of Mexico Research Initiative to the RECOVER Consortium, and in part by CAPES/INCT-TA and CNPq.

RP053 Cross-generational toxicity of polycyclic aromatic hydrocarbons may prolong the ecological impact of oil spill events

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Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous aquatic contaminants that have become an important environmental health concern in part due to oil spill events. While the effects of adult and developmental exposures to PAHs are relatively well characterized, the potential for PAHs to have multigenerational effects remains to be explored. To this end, this study evaluated the cross-generational bioenergetic effects of the model PAH benzo(a)pyrene (BaP) following a chronic maternal exposure using the model teleost Danio rerio. Notably, maternally exposed (ME) F1 individuals exhibited mitochondrial dysfunction and oxidative stress during development at exposure levels that were asymptomatic in the exposed F0 females. The mitochondrial dysfunction persisted later in life in cardiac tissue with reduced mitochondrial reserve capacity, suggesting diminished capacity to respond to increased ATP demand. ME F1 individuals also exhibited significant cardiac hypertrophy, which may reflect

an attempt to compensate for reduced cardiac function. Notably, cardiac function and plasticity are thought to be key determinants of organismal fitness. Therefore, swimming performance and aerobic respiration are currently being evaluated in ME F1 adults. These data suggest that ME to BaP reduces an organism's mitochondrial function and metabolic plasticity that are critical for organismal ecological fitness. The ability to adjust metabolism will be crucial for organisms to effectively respond to a variety of natural and anthropogenic stressors with global change, creating potential for synergistic interactions with other abiotic stressors such as temperature and hypoxia. Further, ME F1 fish exhibited altered locomotor activity throughout life as well as reduced fear and anxiety behaviors as adults. Taken together, these data suggest that low-level exposure to PAHs such as BaP affect organismal physiology and behavior across generations, creating potential for downstream population and ecosystem level effects. These observations highlight the importance of considering multigenerational endpoints in risk assessments and environmental monitoring studies, including in the context of oil spill events. This work was supported by NIEHS T32-ES021432 and P42-ES010356.

RP054 Evidence for competitive inhibition in Cyprinodon variegatus in response to hypoxia and oil exposure as a function of age

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Hypoxia is a common environmental stressor of fishes in the northern Gulf of Mexico. Fish in these habitats were simultaneously exposed to hypoxia and polycyclic aromatic hydrocarbons (PAHs) during the 2010 Deepwater horizon oil spill. Previous research has shown that simultaneous exposures to hydrocarbons and hypoxia have resulted in competitive inhibition between the Hypoxia Inducible Factor and Aryl Hydrocarbon Receptor pathways due to competitive binding of the shared ligand protein Aryl Hydrocarbon Nuclear Transport Protein (ARNT). The objective of this research is to investigate the effect of cross-talk between these two pathways in response to different environmental conditions during early life development in the sheepshead minnow (Cyprinodon variegatus). Post-hatch (4 dpf) and post-larval (8 dpf) sheepshead minnows were exposed to different HEWAF concentrations under a combination of high/low salinity and hypoxic/normoxic conditions. After the initial 48h oil exposure the organisms were transferred to clean, normoxic water until 12 dpf. Cytochrome P450 1A1 (PAH detoxification) and erythropoietin (red blood cell production) gene expression analysis using qPCR was used to determine potential inhibition of defense pathways. The 48h tissue samples of the post-larval developmental stages indicated suppression of both defense pathways in the highest HEWAF concentrations (> 150 ng/mL), which was not observed in the post-hatch developmental stage or under normoxic or low salinity conditions. These results suggest the post-larval developmental stage of sheepshead minnow is the most sensitive stage to hypoxia and PAH exposure. Further investigation into transcriptional effects was performed using RNAseq, these results will also be discussed.

RP055 PAH biomarker levels in deepwater sharks impacted by the Deepwater Horizon Oil Spill from 2011-2016: Evidence of recovery?

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As the largest oil spill in history in U.S.-controlled waters, the Deepwater Horizon (DWH) Oil Spill resulted in extensive contamination of Gulf of Mexico waters. This posed significant health risks to numerous marine wildlife populations, particularly deepwater species residing in offshore waters within and/or adjacent to the primary contamination zone. Given the population-level impacts that have occurred in some wildlife species as a result of chronic exposure to oil constituents from prior oil spills (e.g., Exxon Valdez oil spill), it is critical to monitor the health of Gulf fish to assess the full impacts of the DWH Oil Spill on these animals. Therefore, to address this problem, we have examined biomarkers of exposure to polycyclic aromatic hydrocarbons (PAHs), the most toxic constituents of crude oil, in deepwater Gulf sharks for the past 5 years. Data on the activity of the PAH-metabolizing biotransformation enzymes, cytochrome P450 1a1 (Cyp1a1) and glutathione-S-transferase (GST), biliary concentrations of PAH metabolites, and levels of hepatic lipid peroxidation in 3 deepwater shark species (Squalus mitsukurii, Squalus cubensis, and Centrophorus granulosus) suggest that sharks residing in oil-exposed locations exhibited physiological responses to increased oil exposure up to 2-3 years after the spill occurred. However, more recent data provide evidence for reduced oil exposure as PAH biomarkers levels in sharks collected 4-5 years after the spill have been among the lowest observed in this study. We explore whether reductions in PAH biomarker levels in sharks from oiled sites represent a recovery trend.

RP056 Global transcriptional responses to Deepwater Horizon oil in red drum (Sciaenops ocellatus) embryos

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The Deepwater Horizon (DWH) incident resulted in extensive oiling of the pelagic zone and fouling of shoreline habitats of many commercially important fish species. Exposure to water accommodated fraction (WAF) of oil from the spill is known to result in developmental toxicity particularly cardiovascular defects in pelagic fish species, but little is known about the effects on local fast-developing estuarine fish species. Unraveling the potentially diverse molecular mechanism of oil toxicity is essential for understanding the hazard posed by complex DWH oil mixtures present in the environment. We analyzed the time-course (24, 48 and 72 hpf) transcriptional responses to two types of oil exposures as WAF from a weathered slick oil (2.5%; approximate 10.1 µg/L PAHs) and another from a non-weathered source oil (0.27%; approximate 0.7 µg/L PAHs) in red drum (Sciaenops ocellatus) embryos. Transcriptomic analysis was performed using high-throughput Illumina RNA sequencing (RNA-Seq) on an Illumina HiSeq2500. Short reads were mapped to reference transcriptomes using the Diamond Basic Local Alignment Search Tool (Diamond Blast) and subsequently counted using a custom perl script, and subsequent translation of the RNA ID's to gene ID's was performed using the NCBI database. RNAseq was analyzed using DEseq2, and significantly up-/down-regulated mRNAs were identified. Downstream gene ontology enrichment analysis was performed using the DAVID Bioinformatics Resource. Biological pathway, function, and network analysis was performed using Ingenuity Pathway Analysis software (Ingenuity Systems, Inc.). Hypothesized transcriptomic responses include Ca^{2+}-cycling and cardiac-, ocular-associated genes, AhR-mediated response, steroid biosynthesis, ribosome biogenesis and immune signaling. Comparisons of these pathways with phenotypic responses revealed cardiac function and morphology were impaired at 2.4 µg/L, and craniofacial malformation occurred at 2.2 µg/L by slick oil exposure. This research was made possible by a grant from The Gulf of Mexico Research Initiative. Data are publicly available through the Gulf of Mexico Research Initiative Information & Data Cooperative (GRIIDC) at https://data.gulfresearchinitiative.org.

Complexity Kills the Minnow: Predicting the Ecological Consequences of Complex Pharmaceutical Mixtures

RP057 Global Probabilistic Hazard Assessment of Calcium Channel-Blockers in the Environment

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The global population concentration is increasingly occurring in megacities where access to medicines is increasing more rapidly than waste management infrastructure is implemented. Calcium channel blockers are routinely observed in urbanizing watersheds receiving wastewater discharges of differential quality. We examined the literature to understand the global distribution of calcium channel blockers in influents, effluent discharges, surface waters, and biota. Diltiazem was the most frequently detected calcium channel blocker in urban systems throughout the world, especially the United States, while information was generally lacking from marine ecosystems and other geographic areas, particularly in developing countries. For example, though more than half of the studies reporting calcium channel blockers were from North America, very few observations were available from Latin America and none were identified from Africa. This information was employed to perform probabilistic ecological hazard assessments (PEHA). Based on data availability, we constructed an environmental exposure distribution (EED) of aqueous concentrations of diltaizem reported in literature, and compared this distribution to available ecotoxicity data, a predicted therapeutic hazard value (THV), and a 1 µg/L ecotoxicity threshold. Unfortunately, no studies were observed that examined mixture toxicity of multiple calcium channel blockers. THVs have been previously used to identify surface water and effluent concentrations predicted to result in fish plasma levels equaling the human therapeutic dose (C_{max}). These analyses revealed that diltiazem surface water concentrations in freshwater and marine ecosystems of developed countries fall below the THV (0.73 µg/L) and 1 µg/L. Within the reported wastewater treatment plant diltiazem observations, 11% and 8% of influent and 2% and 1% of effluent diltiazem concentrations are predicted to exceed the THV (0.73 µg/L) and 1 µg/L. However, diltiazem has been observed in fish plasma above human therapeutic doses. Thus, an additional PEHA revealed 17% of the reported fish plasma concentrations are predicted to exceed the human therapeutic dose (30 µg/L). These findings highlight the utility of global assessments of environmental contaminants to identify regions of additional environmental monitoring, assessment and management, particularly of complex pharmaceutical mixtures.

RP058 Effects of carbamazepine exposure on the whole transcriptome of the model plant Arabidopsis thaliana

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Irrigation with treated wastewater and soil amendment with biosolids are increasingly common practices in agricultural systems, and result in exposure of crop plants to pharmaceuticals. Crop plants may accumulate these contaminants in edible tissues, but prediction of uptake relying solely on physico-chemical properties of the compounds can be confounded by metabolism in planta or the influence of one contaminant on the accumulation of another. In humans, some pharmaceuticals interfere with the efficacy of others. An example of this is the anti-seizure drug carbamazepine, which up-regulates several important drug-metabolizing enzymes. Carbamazepine is frequently detected in treated wastewater and is taken up by and accumulates in plants. Using whole transcriptome profiling, we assessed the effects of carbamazepine exposure on gene expression in the model plant Arabidopsis thaliana. We hypothesized that enzymes homologous to those up-regulated by carbamazepine in humans would also be up-regulated by carbamazepine in plants. We grew A. thaliana hydroponically and exposed roots to 1 µg/L or 100 µg/L carbamazepine for 24 hours. After exposure, we harvested leaf tissue and extracted RNA from exposed and unexposed control plants. We performed whole transcriptome profiling using the extracted RNA and Affymetrix gene expression microarrays, and compared mRNA expression levels between treatments for genes encoding drug-metabolizing enzymes and other proteins. We determined carbamazepine concentrations in leaf tissue by extracting additional plants from each treatment using accelerated solvent extraction followed by quantification using HPLC-MS/MS. Compared to the control, 8 genes were up-regulated and 42 genes were down-regulated in the 1 µg/L carbamazepine treatment, and 15 genes were up-regulated and 33 genes were down-regulated in the 100 µg/L treatment. The implication of these results on the accumulation of other pharmaceuticals will be discussed.

RP059 Altered Development: The effects of early venlafaxine (Effexor) exposure on zebrafish

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The rise of antidepressant prescriptions worldwide has resulted in an increased release of these neuroactive compounds into aquatic ecosystems. Venlafaxine (Effexor) is one of the most highly prescribed antidepressants worldwide, resulting in levels of the drug in the µg/L range in our waterways. As antidepressants are often designed to function at low doses, there is growing concern over how these drugs will affect non-target species that often share evolutionary conserved pathways with humans. Venlafaxine acts as a serotonin and norepinephrine reuptake inhibitor in humans, suggesting that key systems such as the brain and heart may be impacted during development, adversely affecting the survival of developing organisms. This study assesses changes in the developmental profile of zebrafish (Danio rerio) exposed to venlafaxine immediately after fertilization. Zebrafish embryos were injected with venlafaxine at the 1-4 cell stage, and assessed for behaviour, neurogenesis, and cardiac performance. Embryo exposure to this drug caused precocious development, including increased hatch rate and larger larvae at 5 days post fertilization (DPF). Venlafaxine increased neurogenesis and affected behavior, including a dose-dependent reduction in the escape response of larval zebrafish at 5DPF. The drug also affected larval cardiac performance. Overall, embryo exposure to venlafaxine disrupts larval development and performances in zebrafish. This study was supported by the Natural Sciences and Engineering Research Council of Canada Discovery and Strategic Grants to MMV and an Eyes High Doctoral Scholarship to WAT.

Differing Biotransformation Capacity Across Species: Measurements, Modeling and Implications for Decision-Making

RP060 The Bioaccumulation Assessment Tool: An Organizational Framework for Bioaccumulation Assessment

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Thousands of chemicals are under evaluation for potential hazard, exposure and risk to ecological receptors and humans. Screening methods include the application of Persistence, Bioaccumulation and Toxicity (PBT) criteria against available data. A variety of regulatory programs require Bioaccumulation assessment, e.g. Europe's REACH legislation, the Canadian Environmental Protection Act (CEPA), and Japan's Chemical Substances Control Law. The multitude of metrics for assessing bioaccumulation include: the octanol-water partition coefficient (K_{OW}); the bioconcentration factor (BCF) determined under controlled laboratory conditions (e.g., OECD 305); the biomagnification factor

(BMF) determined in the laboratory (e.g., OECD 305), or measured in the environment; the bioaccumulation factor (BAF) measured in the environment; the trophic magnification factor (TMF) measured in the environment; and the total elimination half-life (HL). Additionally, there are various quantitative criteria and quantitative and qualitative thresholds for the previously listed bioaccumulation metrics, e.g., REACH Annex XIII; however, there are no well-defined implementation strategies that include all of these lines of evidence. The objective of this research is to develop a user-friendly organizational framework and computational tool in the form of an Excel/VBA spreadsheet for integrating various lines of evidence in a consistent and transparent quantitative weight of evidence (QWOE) approach to guide bioaccumulation assessment decision-making. The Bioaccumulation Assessment Tool (BAT) brings together measured and modelled data, e.g., chemical properties, in vivo data (BCFs, BMFs, HL, absorption efficiency), in vitro data (intrinsic hepatic clearance rate) and in silico data (BCF-QSPRs, biotransformation rate constant QSPRs). These multiple lines of evidence are treated as "Input". Primary "Output" from the BAT includes a suite of bioaccumulation assessment endpoints presented against user-defined criteria/thresholds and data for benchmark chemicals, enabling the assignment of bioaccumulation classifications (e.g., "B" or "not B"). The BAT will enable transparent and consistent bioaccumulation assessment and guidance for integrated (tiered) testing strategies to address uncertainty. The development of the BAT will include multi-stakeholder participation and final versions of the BAT will be freely available.

RP061 Integration of Important Processes into a Novel Spatial Aquatic Food-Web Model for Accurate Assessment and Measurement of Chemical Bioaccumulation

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A novel, complex food-web model has been developed to accurately assess and measure chemical bioaccumulation in real aquatic systems by incorporating important processes such as spatial heterogeneity, species migration, and field sampling design into the existing AQUAWEB model. The new Multibox-AQUAWEB (MBAW) model allows users to define properties of numerous sub-compartments by specifying vertical and/or horizontal concentration gradients in an aquatic system. The model also requires the users to define species composition, structure, and trophic dynamics of the aquatic food web. For species migration, the users can define the fraction of time that each species occupies a particular compartment. This input specifies the distribution of a species to a certain area and the degree to which a species may be present in multi-dimensional space. The model also provides the users with the option to specify the "sampling" location of each species by identifying the compartments from which the species will be collected. Using the inputs and mechanisms in the model, the MBAW model calculates the steady-state (whole body wet weight and lipid-equivalent) chemical concentrations in species in each compartment. Based on the predicted concentrations, trophic magnification factors (TMFs) as well other conventional bioaccumulation metrics such as BCF, BAF and BSAF are calculated. TMFs can be also calculated for various sampling scenarios to investigate the effect of sampling design on the determination of the TMF in areas with significant spatial concentration gradients. The MBAW model provides guidance on both the conduct and interpretation of field bioaccumulation studies and highlights the need for development of detailed protocols for field bioaccumulation studies in aquatic food-webs.

RP062 In vitro to in vivo extrapolation of hepatic metabolism in fish: an inter-laboratory comparison of in vitro methods

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Chemical biotransformation represents the largest source of uncertainty in chemical bioaccumulation assessments. Model-based estimates of chemical bioconcentration in fish may be greatly improved by including biotransformation rates, as measured in vitro. Substrate depletion assays with trout hepatocytes or liver subcellular fractions (S9) have been successfully employed to this end. Building on previous work, a multi-laboratory ring trial was coordinated by the ILSI Health and Environmental Sciences Institute (HESI). The specific aims of the ring trial were to determine the reliability of these assays within and across laboratories, compare the performance of substrate depletion assays using the two biological systems, and support the development of two OECD test guidelines (OECD Project 3.13). Six laboratories conducted substrate depletion assays for 6 test chemicals (pyrene, 4-n-nonylphenol, fenthion, cyclohexyl salicylate, deltamethrin, methoxychlor) using both trout liver S9 fractions and trout hepatocytes to determine in vitro intrinsic clearance ($CL_{in\ vitro,int}$). Using either test system, participating laboratories measured similar rates of metabolism for each test chemical, and the rates for each chemical exhibited similar variability. The intra-laboratory agreement for each test chemical in $CL_{in\ vitro,int}$ averaged 15-23 %CV for the hepatocyte assays and 5-29 %CV for the S9 assays. The inter-laboratory agreement was somewhat less, ranging from 22-40 %CV for the hepatocyte assays and 10-29 %CV for the S9 assays. Variability in predicted hepatic clearance values, and by extension modeled BCF estimates, was lower than variability exhibited by the in vitro data sets due to blood flow limitations on clearance. For all test chemicals, hepatic clearance values determined by the two test systems were in good agreement (within 2-fold). Bioaccumulation predictions generated using the in vitro data had closer agreement with empirical BCF values than traditional modeled estimates, even for the more slowly metabolized compounds. Results of this ring trial firmly establish the reliability of these methods and provide strong support for use of this information in modeled BCF assessments for fish.

RP063 Metabolism of fragrance materials using trout in vitro metabolism assays

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Bioconcentration data for many chemicals including fragrance materials still is scarce and much needed to determine bioaccumulation potential. There is an increasing global interest in the presence of fragrances in the environment due to fact that selected fragrance ingredients (e.g., synthetic musks) and their metabolites have been found at relatively low concentrations in a variety of aquatic environmental environments. There are several factors that pose significant challenges with the approach of assessing bioaccumulation using in silico models or extrapolation from measured log Kow (partition coefficient). Fragrance ingredients are comprised of many unique chemical classes (i.e., macrocyclic lactones and lactides, thiols, cyclic terpenes, hydroquinones, etc.) that are often not well represented in many of the common in silico databases upon which these environmental models (e.g. the USEPA's Estimation Program Interface Suite) are based and therefore, leading to over prediction of fragrance ingredient bioconcentration potential. Secondly, while it is well-established that BCFs can be reasonably predicted from log Kow values for neutral organics of intermediate lipophilicity (i.e., log Kow < 6), some fragrance ingredients may exceed this level and the determination of their experimental log Kow values (and subsequent BCF predictions) may be highly uncertain due to experimental errors. Finally, the determination of simple octanol-water partition coefficients do not take into account the potential for metabolism of the test chemical which has been shown to significantly decrease the amount of estimated passive bioaccumulation of highly lipophilic chemicals. The purpose of the present study was to determine metabolic stability in trout liver S9 fractions as well as in cryopreserved hepatocytes of various fragrances with Kow values >4. The results indicate that 19 out of the 21 fragrances tested were significantly metabolized by rainbow trout liver S9 fractions and hepatocytes. In vitro metabolism of chemicals such as those used in the present study support the conclusion that measured Kow values >4 are not always predictive of high BCF values. Liver S9 fractions and cryopreserved hepatocyte incubations are considered additional powerful tools to assess bioaccumulation potential of chemicals.

RP064 Concentration dependence on in vitro biotransformation rates: Hepatic biotransformation of organic sunscreen agents in rainbow trout

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In vitro bioassays to estimate chemical biotransformation rate constants in fish are currently being investigated to improve bioaccumulation assessments of hydrophobic contaminants. The effect of concentration dependence on in vitro biotransformation rates (k_{dep}) has been recently illustrated. It's suggested that measuring k_{dep} initial substrate concentrations of 1 μM (a current convention) could underestimate the in vitro biotransformation potential and may cause bioconcentration factors (BCFs) to be overestimated if k_{dep} is used to assess BCFs in fish. Evaluating the concentration dependence on k_{dep} may also be important when no Michaelis Menten kinetic information exists for chemicals of interest. In this study multiple solvent delivery-based depletion experiments at a range of initial concentrations were conducted to investigate the concentration dependence on k_{dep} of organic ultraviolet filters (UVFs). UVFs are ingredients common in sunscreens and personal care products and may enter the aquatic environment directly from the skin during recreational activities in water or indirectly via wastewater effluents. Many UVFs are hydrophobic ($logK_{OW}$ > 4.0) and their bioaccumulation in higher trophic level organisms and in aquatic food webs in the field has been observed, suggesting that further investigations evaluating UVF bioaccumulation potential are warranted. The present study measured k_{dep} of selected UVFs: avobenzone, 4- methylbenzylidene camphor, ethylhexyl trimethoxycinnamate, and octocrylene ($logK_{OW}$s ranging from 4.5 to 6.9). The results illustrated that the relationship between k_{dep} and substrate concentration is well described by the Michaelis-Menten equation. Michaelis-Menten constants and maximum biotransformation rates are reported. Kinetic parameters were then incorporated into an in vitro-in vivo extrapolation (IVIVE) model to predict a BCF for UVFs. BCFs predicted by the model were then compared to: in vivo (empirical) BCFs; BCFs predicted from biotransformation rates estimated using a QSAR model; and BCFs predicted without biotransformation. We discuss the importance of considering the concentration dependence of biotransformation rates in the IVIVE of biotransformation data for bioaccumulation assessment.

RP065 Concentration dependence of in vivo biotransformation rates of organic sunscreen agents in rainbow trout following a dietary exposure

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Simple diffusion lipid-partitioning models have historically described the bioaccumulation of hydrophobic chemicals in fish. While these models are sufficient to describe the bioaccumulation of recalcitrant PCBs and other non-metabolized chemicals, they are inadequate for chemicals that are biotransformed. Consequently, efforts have been made to improve bioaccumulation models by incorporating estimated chemical biotransformation rates. While bioaccumulation models have become more sophisticated, they continue to assume that chemical uptake and elimination are independent of chemical (exposure) concentration, although this assumption has been scarcely investigated. The concentration dependence of in vitro hepatic biotransformation rates has been recently illustrated and is an important consideration for in vitro to in vivo extrapolation (IVIVE) of biotransformation data. Assays performed at substrate concentrations exceeding the Michaelis Menten affinity constant (Km) may underestimate a chemical's intrinsic clearance rate, potentially resulting in underestimates of in vivo hepatic clearance and overestimates of modeled bioaccumulation. These outcomes depend, however, on the relationship between tested in vitro concentrations and the in vivo concentration achieved the liver (C_{liv}). In this study, we investigated the relationship between dietary exposure concentration and observed accumulation and elimination of two organic ultraviolet filters (UVFs): ethylhexyl trimethoxycinnamate and octocrylene (log Kow of 5.8 and 6.9, respectively). Three dietary concentrations of UVFs covering 2 orders of magnitude were selected to result in a range of anticipated C_{liv} values. Rainbow trout were exposed over a 14d uptake period and 14d depuration period. Fish were collected during the 28d exposure and tissues (plasma, liver, gastrointestinal tract, carcass) were extracted and analyzed for chemical concentration. Fish were simultaneously exposed to non-biotransformed reference chemicals. The decline in chemical concentration over the depuration period was compared between the UVFs and reference chemicals to estimate k_{MET} for the UVFs. The influence of varying dietary concentrations on k_{MET} was then evaluated. Data generated from this test will be used to inform IVIVE approaches in bioaccumulation assessment.

RP066 Differences in detoxification pathways between sunfish and chub in relation to sensitivity to environmental pollutants

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Observations in a previous study on biomarker responses in fish collected from urban creeks in Greenville, SC, indicated that there might be considerable differences in the expression of biotransformation enzymes in chub and sunfish species. To further investigate these species differences, a dosing experiment was performed in which bluehead and creek chub (Nocomis leptocephalus and Semotilus atromaculatus), and redbreast sunfish and bluegill (Lepomis auritus and L. macrochirus) were injected with benzo[a]pyrene (BaP) as a model compound for common pollutants in urban creeks. Fish were injected with BaP doses of 0, 25 and 50 mg/kg, and after 3 days BaP metabolites in bile, and enzymatic activities of cytochrome P450-1A (CYP1A), UDP-glucuronosyltransferase (UGT) and glutathione S-transferase (GST) were measured. CYP1A activity was significantly increased after BaP dosing in both species groups, but in chub were much lower than the levels that were observed in the dosed sunfish. The UGT activity in unexposed animals was comparable in both species groups, and was significantly increased in both groups as a result of BaP dosage. Finally, GST activity was not changed in either species group as a result of BaP exposure. However, the results confirmed that while unexposed chubs have much lower CYP1A activity than sunfish, they have a much higher GST activity than sunfish. The metabolized BaP was excreted in bile of both species groups, but at the time of sampling there were no clear differences in the amount of BaP metabolites in the bile of dosed animals, despite the differences in enzyme expression. The differences in baseline enzyme activity and induction capacity between both species groups may explain why chubs could be more sensitive to exposure to environmental pollutants than sunfish. This conclusion was corroborated by the observation of significant mortality in the chubs at the highest BaP dose of 50 mg/kg, which was obviously close to the apparent LC50 for chub, while no mortality was observed in the sunfish at this dose. These species differences in expression and regulation of biotransformation enzymes should be considered when implementing biological effect monitoring programs.

RP067 Correlation of Contaminant Levels in Whole Body and Fillet Tissue Samples: Application of a Simple Lipid-Based Model across Multiple Contaminants

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A multi-year fish sampling program was conducted to support characterization of human health and ecological risks associated with contaminant exposure in the Lower Passaic River. Despite these efforts, not all fish tissue types needed for the risk assessments conducted for the lower 8.3-mile Focused Feasibility Study were obtained. Specifically, two additional tissue types were needed: whole body for white perch for ecological risk estimates and fillet for American eel for human risk estimates. Adjustment factors were developed to estimate these tissue concentrations for contaminants of concern using a larger set of co-located complimentary tissue types (fillet for white perch and whole body for American eel). The objective was to develop statistically supported factors to estimate whole body concentrations for white perch when only fillet samples were available, and to estimate fillet concentrations for American eel when only whole body samples were available. These factors were needed for a number of high K_{oc} compounds, whose concentrations in fish tissue are generally well correlated with tissue lipid content. To help reduce uncertainty in estimating concentrations for one tissue type from the other, the adjustment factors were developed based on lipid-normalized concentrations, which helped account for concentration differences between sample tissue types that are related to lipid content differences. The analysis considered 7 contaminants, including 2,3,7,8-TCDD, Total PCBs, DDT, dieldrin, chlordane, low and high molecular weight PAHs. For both adjustment factors, 95 percent uncertainty bounds based on bootstrap analysis of the uncertainty were + 22 percent of the value of the factor or less, indicating a strong predictable relationship between co-located samples of differing tissue types for the same species. For American eel, the adjustment factors of lipid-normalized concentrations (whole body to fillet) for these compounds yielded a mean value (1.1) with a range across the 7 compounds of 0.87 to 1.4. The adjustment factors of lipid-normalized concentrations (whole body to fillet) in white perch for these compounds yielded a mean value of 1.6, with a range of 1.2 to 2.0 across the compounds. The lipid-based adjustment factor approach using similarly exposed animals (co-located samples) for the 7 compounds provided a basis to develop estimates of the missing tissue concentrations for use in the risk assessments, with well characterized uncertainty.

RP068 Liver CYP1A expression induced by benzo(a)pyrene and its relation with biochemical and genotoxic biomarkers in a Neotropical fish

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The goal of this work was to study the expression of CYP1A gene in the Neotropical fish Prochilodus lineatus and its relation with phase I (EROD) and phase II (GST) biotransformation enzymes, the content of glutathione (GSH), and oxidative (LPO) and genotoxic damage after a single injection of benzo(a)pyrene (B(a)P). Juveniles of P. lineatus received one intraperitoneal injection of B(a)P (20 mg kg^{-1}) dissolved in canola oil (B(a)P group) or an equal volume (canola oil: 2 mL kg^{-1}) of the vehicle (OIL group). After injection, fish remained for 6, 24 and 96 h in clean water. At each experimental period blood samples were collected for the comet assay and fish were killed for the removal of the liver for RT-qPCR (using specie-specific primers), biochemical analyses (EROD, GST, GSH, LPO) and comet assay. B(a)P fish showed CYP1A gene expression in the liver significantly higher than OIL fish, at all experimental periods (6, 24 and 96 h). After 24 h, B(a)P fish showed the highest increase (18 x) in CYP1A gene expression. The hepatic activity of EROD increased significantly at the three experimental periods. Thus, enzyme activity (EROD) and the expression of the correspondent gene (CYP1A) increased after 6 h of B(a)P injection and remained elevated for 96 h. Despite the higher activity and expression of CYP1A, GST activity did not show any significant change suggesting the involvement of other biotransformation pathways. However, after 6 h of B(a)P injection, there was a significant decrease in GSH concentration , suggesting the consumption of GSH as antioxidant and the involvement of other antioxidant defenses, as no increase in oxidative damage (LPO) was observed at any period. Significant increases in DNA damage were observed in B(a)P fish, at 6 h period, both in the liver and red blood cells. As intermediate metabolites of B(a)P can be toxic and more reactive than the non-metabolized compound, these reactive intermediates would have caused this genotoxic effect in liver. DNA damage in blood cells supports this idea and suggests that B(a)P metabolites, produced in the liver, were transported through the bloodstream. The expression of CYP1A gene and its relation with the corresponding enzyme activity (EROD) are important tools to understand the response mechanisms of P. lineatus exposed to organic compounds. The results of GST, GSH and LPO indicate that other biotransformation pathways and antioxidant defenses are involved in B(a)P metabolism in P. lineatus.

RP069 In vitro and in silico assessment of cytochrome P450-dependent biotransformation capacity of PCBs in the Caniformia; the Baikal seal vs the beagle dog

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Biotransformation of polychlorinated biphenyl (PCB) congeners is initiated by cytochrome P450s (CYPs) in a species- and CYP isozyme-specific manner. Our previous study revealed that the Baikal seal (Pusa sibirica) CYP1A1 has a low capacity for the biotransformation of PCBs, whereas CYP1A2 lacks the capacity due to the Pro substitution of highly conserved Thr in the I-helix. The beagle dog (Canis lupus familiaris) has been suggested to have a high CYP-dependent biotransformation capacity of PCBs. However, we still do not know which CYP isozymes are involved in the biotransformation of individual PCB congeners in the Caniformia including the seal and dog. We thus assessed the CYP-dependent biotransformation capacity of 62 PCB congeners by in vitro assays using the liver microsomes of the Baikal seal and the beagle dog and by in silico docking simulations of these PCBs with their CYP isozymes. In the liver microsome of wild Baikal seals, a decreased ratio of over 20% to loaded PCB congeners was observed for 10 of mono- to penta-chlorinated congeners, suggesting the preferential metabolism of low chlorinated PCBs by seal CYPs. As for the beagle dog, 35 PCB congeners with mono- to nona-chlorine substitutions were metabolized even in the liver microsome of non-PCB-treated dogs; constitutively expressed CYPs like CYP2A, 2C and 3A may be involved in PCB metabolism. In the liver microsome of dogs treated with 12 PCB congeners by which CYP1A1 and 1A2 levels were induced, only a few congeners like CB28 and CB123 were more metabolized than in non-PCB-treated dogs. This indicates that dog CYP1A1 and 1A2 participate in the biotransformation of only limited congeners. In the liver microsome of dogs treated with a mixtutre of phenobarbital and 12 PCB congeners by which CYP2B11 levels as well as CYP1A1 and 1A2 were induced, increased metabolism of 12 penta- to nona-chlorinated congeners was observed compared with the result of PCB-treated dogs, implying their metabolism by CYP2B11. We then constructed in silico homology models of seal CYP1A1, 1A2, 1B1, 2A, 2B, and 2C, and dog CYP1A1, 1A2, 1B1, 2A25, 2B11, 2C21, and 3A12, and simulated their docking with PCB congeners. The principal component analysis revealed a major contribution of CYP2A and 2B to PCB biotransformation in both seals and dogs. This study demonstrates that the combination of in vitro and in silico analyses is a useful tool to assess the biotransformation capacity of PCBs by each CYP isozyme.

Alternative Approaches to Complex Environmental Challenges

RP070 The Evaluation of Multiple Milkweed Species: Feasibility of Use in Standard Plant Toxicity Test Designs and Sensitivity to a Common Herbicide

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On May 19, 2015 the Office of Science and Technology Policy released President Obama's interagency task force's Strategy to Promote the Health of Honey Bees and Other Pollinators. A part of that strategy is the U.S. Environmental Protection Agency's (EPA) commitment to the protection of the milkweed plants, the sole food source and significant habitat for the monarch butterfly (Danaus plexippus). Five commercially available milkweed species: Butterlfy Weed (Asclepias tuberosa), Common Milkweed (Asclepias syriaca), Swamp Milkweed (Asclepias incarnate), Whorled Milkweed (Asclepias verticillata) and Bloodflower (Asclepias curassavica) will be evaluated for their feasibility of use in the seedling emergence and vegetative vigor test formats outlined in the OCSPP 850.4100 and 850.4150 guidelines. The results pertaining to percent emergence, percent survival, shoot length, and shoot dry weight will be provided for each species. Previous work completed determined that Bloodflower (Asclepias curassavica) is suitable for testing as it meets the referenced guideline acceptability criteria. Based on the results of the feasibility trials, seedling emergence and vegetative vigor tests will be performed to evaluate the sensitivity of any suitable milkweed species to Glyphosate, the widely used broad spectrum herbicide. Endpoint sensitivity comparisons will be made across the milkweed species utilized and potentially compared to other standard crop species used in non-target plant testing.

RP071 Sensitivity of zebrafish larvae behaviour in the context of on-line contamination event detection in water distribution systems

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An effective biological early warning system (BEWS) for the detection of water contamination should employ undemanding species that rapidly react to the presence of contaminants in their environment. BEWSs can provide more reliable predictions about substance toxicity than the chemical concentration alone, especially in case of mixtures of substances. This allows for the detection of potentially adverse biological effects in cases where traditional detection methods fail or are too costly. Here, we propose to use behavioural changes of zebrafish larvae for the detection of sudden contamination events in a water stream. In order to test for the sensitivity and rapidity of the response, zebrafish larvae at the age of 96 hours post fertilization (hpf) were exposed to cadmium chloride, permethrin, ethanol and sodium hypochlorite in low concentrations and immediately monitored for behavioural changes. For the analysis of zebrafish behaviour data we propose two approaches that have the potential to be used for the evaluation of on-line data streams. For the first approach data on the distance larvae moved was pooled within groups (control n=16; 4 treatment groups at different concentrations with n=20 each) and averaged for one minute per group. In this approach two upper thresholds for maximal activity of the larvae during darkness were defined (180 mm/min and 200 mm/min). For the second approach the x- and y-coordinates of the larvae's movement where transformed to polar coordinates in order to calculate the angle of their movement. Thresholds for the angle allow distinguishing between undirected movement of the larvae and directed circular swimming behaviour that was defined as avoidance behaviour. First results show that the approaches are promosing methods to detect alterations in zebrafish larvae behaviour in response to low concentrations of the investigated contaminants. The thresholds presented in this study are sensitive with a minimum in false positive alerts and have the potential to be used for the analysis of an on-line data stream. The second approach considers the directed behaviour of the larvae and therefore allows for the detection of a distinct parameter. Overall, the zebrafish larvae behaviour appears to be a sensitive and rapid endpoint that could be considered for the implementation in water monitoring.

RP072 Determining aggregation behavior and corresponding surface reactivity of copper oxide nanoparticles using a rapid colorimetric assay

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The ability to predict interactions with abiotic or biotic surfaces in complex systems would enhance our understanding of the life cycle of nanoparticles (NPs) and assist in evaluation of risk. The aggregation behavior of NPs is a key process determining fate, transport, and bioavailability and is dictated by environmental interactions. In this study, we propose a method to evaluate NP homoaggregation and heteroaggregation, as measured by changes in surface reactivity. Methylene blue dye (MB) was used as a chemical probe to observe the reactivity of NPs used to catalyze the reduction of MB by sodium borohydride. The resulting color change was measured with ultraviolet-visible spectrophotometry. We hypothesized that the rate of reaction is directly related to the NP aggregation state. Copper oxide (CuO) NPs were selected as a model NP in this study due to their widespread industrial and commercial applications and their high reactivity. The relative reactivity of 1 mg/L CuO was evaluated after CuO NPs were allowed to either homoaggregate for 2 and 24 hours or heteroaggregate with green algae Chlorella vulgaris for 24 hours. The resulting change in surface area for homoaggregated CuO NPs was evaluated using nanoparticle tracking analysis (NTA). The reaction rate did not significantly decrease after 2 hours of homoaggregation. However, the rate significantly decreased by 80% after 24 hours homoaggregation compared to non-aggregated CuO NPs, and by 34% when CuO NPs were heteroaggregated with algae cells. The decrease in reaction rates corresponded to a decrease in overall surface area from nm^2/mL for non-aggregated particles to nm^2/mL and after 2 and 24 hours of homoaggregation, respectively. This method allows us to rapidly and efficiently assess both surface reactivity and aggregation and can be applied to environmentally relevant complex suspensions.

RP073 Pesticides and public health

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Pesticides are designed to kill and because their mode of action is not specific to one species, they kill or harm organisms other than pests. The application of pesticides is often not precise, unintended exposure occur to other organisms in the areas where pesticides are applied. Children and indeed developing organisms are particularly vulnerable to the harmful effects of pesticides. Even very low levels of exposure during development may have adverse health effects. Pesticide exposure can cause a range of neurological health effects such as memory loss, loss of coordination, response to stimuli, reduced visual ability, uncontrollable mood and reduced motor skills. These symptoms are often very subtle and may not be recognized by the medical community as a clinical effect.Other possible health effects include asthma, allergies, hypersensitivity, cancer, hormone disruption, reproduction and fetal development. Pesticide formulations contain active and inert ingredients. Active ingredients are what kill the pests, inert ingredients help the active ingredients to work more effectively. These inert ingredients may not be tested as thoroughly as active ingredients, seldom disclosed on product labels. Solvents are inert ingredients in in many pesticide formulations may be toxic if inhaled or absorbed by the skin. Children are at greater risk from exposure to pesticides because of their small size: children eat, drink, breathe more than adults, they may be exposed to pesticides even while in the womb. In the light of the foregoing, it should be noted that pesticides are not the only way to keep pest away. There are many alternative repellents and pesticides as well as other natural methods such as biological control of pest. Pesticides present the only group of chemicals that are purposely applied to the environment with aim to suppress plants and animal pest and to protect agricultural and industrial products. However, the majority of pestsicides is not specifically targeting the pest only,they also affect non-target organisms. Overall, intensive pesticide application results in several negative effects in the environment that cannot be ignored.

RP074 ECETOC/RIFM WS: Developing a strategy to improve the environmental risk assessment of difficult to test multi-component substances Part 1: Background

D.T. Salvito, Research Inst for Fragrance Materials, Inc. / Dept of Environmental Science; M. Galay-Burgos, ECETOC

An international workshop was held on 2 – 4 November 2016 to address challenges in risk assessing complex mixtures of substances (e.g., multi-constituent substances (MCS), unknown variable composition and biological substances (UVCBs)). International regulatory schemes (specifically REACH, Environment Canada's DSL Categorization, and USEPA's PMN process) have highlighted the complexities of registering, characterizing fate and exposure, and risk assessing complex chemical mixtures whether from manufacturing environments or plant derived materials. Several industrial sectors (e.g., petrochemicals, personal care) have developed schemes for characterization and analysis of these complex substances. This Workshop was designed to identify best practices and key research needs to support environmental risk assessment. Reported here is the background leading up to this Workshop.

RP075 ECETOC/RIFM WS Developing a strategy to improve the ERA of difficult to test multi-component substances Part 2: Recommendations

D.T. Salvito, Research Inst for Fragrance Materials, Inc. / Dept of Environmental Science; M. Galay-Burgos, ECETOC

An international workshop was held on 2 – 4 November 2016 to address challenges in risk assessing complex mixtures of substances (e.g., multi-constituent substances (MCS), unknown variable composition and biological substances (UVCBs)). International regulatory schemes (specifically REACH, Environment Canada's DSL Categorization, and USEPA's PMN process) have highlighted the complexities of registering, characterizing fate and exposure, and risk assessing complex chemical mixtures whether from manufacturing environments or plant derived materials. Several industrial sectors (e.g., petrochemicals, personal care) have developed schemes for characterization and analysis of these complex substances. This Workshop was designed to identify best practices and key research needs to support environmental risk assessment. Reported here are the recommendations for best practices and areas for further research developed at the Workshop.

RP076 Screening Assessment for Unique Human Health Exposure Pathways

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USEPA and various states have developed health-based screening levels for various media (soil, water, air) which risk assessors commonly use to identify chemicals of interest (COI). Analytes with concentrations below corresponding health-based screening levels are not expected to pose significant risk and are subsequently not carried forward to the baseline risk assessment. In cases of uncommon exposure pathways, the absence of risk-based screening levels can result in the inclusion of all detected concentrations. The consumption of game meat potentially impacted by contaminated site media may pose a risk to human health. In the absence of game meat health-based screening levels, analytes detected in the various media may be considered a COI and carried forward in the baseline risk assessment. USEPA has acknowledged that screening based on risk contribution can greatly reduce the number of chemicals carried through a risk assessment. USEPA notes that "in many cases, only a few chemicals contribute significantly to the total risk for a particular medium." A modified screening evaluation was designed to reduce the number of COIs by eliminating chemicals that are not anticipated to contribute significantly to the baseline risks. The modified screening evaluation models the game meat exposure point concentration (EPC) and screens it against soil screening levels to determine relative risk contributions. Any compound with a relative risk contribution less than 1% of the total cancer or non-cancer ratio sum was eliminated as a COI. Of the 65 analytes detected in soil and/or surface water, 9 had maximum soil concentrations below background concentrations. Based on this modified screening approach using relative risk contributions, 47 analytes were eliminated. A total of 31 analytes contributed 0.4% of the total non-cancer hazard and 7 contributed 0.03% of the overall cancer risk. Of the remaining 18 analytes, 8 COIs contribute 0.1-80% of the non-cancer HI and 10 COIs 0.1-70% of the cancer risk. By applying a modified screening based on relative risk contribution, the list of COIs was reduced by over 70%, and the risk assessment avoided inclusion of analytes that are expected to pose insignificant risk.

RP077 Silicone wristbands detect occupation and lifestyle differences in chemical exposure in rural Peru

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It is a challenge to assess human exposure to complex mixtures of chemicals. Especially residents of developing regions can bear substantial chemical exposure due to poorly enforced regulations or education about safe practices. The Alto Mayo, in Peru, is a prototypical region with developing agriculture and potential health impacts on its residents are largely uncharacterized. The identification of the top chemicals in their environment and what groups may be most at risk is an important component of a comprehensive health assessment. Silicone wristbands are a novel passive sampling device for measuring lipophilic chemicals in the personal environment of people and are a promising technique for use in remote areas. In the current study, 69 volunteers from four communities of the Alto Mayo wore wristbands for 31-34 days. We screened the wristbands for 1418 compounds with gas chromatography and mass spectrometry coupled with deconvolution reporting software and we quantitatively measured 63 pesticides by gas chromatography-electron capture detection. We detected 106 unique compounds from eight chemical classes. Several chemical classes were associated with demographic variables, indicating an effect of occupation and lifestyle on chemical exposure. Some highlights include that personal care products were more frequent in wristbands worn by female participants than males ($p = 0.0004$) and different between communities ($p < 0.0001$). The frequency of PAHs and flame retardants were also different among demographics of the Alto Mayo. The presence of fungicides and insecticides were significantly associated with community and whether the participants were farm workers. Quantitative pesticide results showed that chlorpyrifos was one of the chemicals driving those differences. Chlorpyrifos was the most commonly detected pesticide and was measured from 17 to 9000 ng/g wristband. After accounting for other factors, chlorpyrifos concentrations were significantly different between communities and occupations. These results suggest a chemical signature associated with lifestyle, and could be used to identify groups of people that are most at risk for adverse health effects from specific chemicals. This is the first study to use wristbands to examine, across multiple chemical classes, differences in exposure between demographic groups, demonstrating a promising approach to investigating personal exposure to many chemicals, especially in developing regions.

RP078 Application of Bioavailability Corrections to Develop Site-Specific Toxicity Thresholds for Copper, Lead, and Zinc, at the Hanford Nuclear Site

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A group of eight State and Federal agencies and Tribes are participating in a Natural Resource Damage Assessment (NRDA) at the Hanford Nuclear Site in Eastern Washington. The Site has more than 40 contaminants of potential concern (COPCs) within a 1500 km^2 area, including portions of the Columbia River. For the purposes of injury determination, the Natural Resource Trustees rely upon thresholds, with exceedance of these values informing the development of a case for injury. We reviewed multiple available thresholds including promulgated regulatory standards, criteria, and literature values. The narrative intent of these thresholds varied depending on medium and jurisdiction, some of the reported values come from a single, sensitive organism or study (e.g., NOAEL, LOAEL, Benchmarks), or represent conservative screening values (e.g., Eco-SSLs), or were derived from species sensitivity distributions (e.g., Water Quality Criterion), and as such there was a wide range of values (e.g., 0.05 to 323,000 µg/L for lead in surface water). In addition to the wide range, these values may not adequately reflect injury to ecological receptors within the unique Hanford shrub-steppe ecosystem. In this poster we describe in detail the application of bioavailability corrections to develop site-specific injury thresholds for copper, lead, and zinc, in soil, surface water, and sediments, as part of the iterative process adopted by the Trustees to assess injury. We used multiple empirical approaches to inform our NRDA evaluation of these COPCs, notably a soil Predicted No-Effect Concentration (PNEC) calculator (ARCHE), the Biotic Ligand Model (WindWard), Consensus-Based Sediment Quality Guidelines, AVS/SEM, and Equilibrium Partitioning approaches. Many of these approaches have been successfully applied in environmental risk assessments, and are becoming more common in NRDAs. The benefits and challenges of these approaches will be discussed, and the results compared to promulgated thresholds, existing ecotoxicity literature, and Hanford specific ecotoxicity studies. Incorporating these multiple approaches has strengthened our ability to understand uncertainty, and aided in the decision making process.

RP079 Risk Assessment of Occupational Exposure to Polycyclic Aromatic Hydrocarbons (PAHs) and Metals in Firefighters

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Firefighters experience elevated risk of injury and developing chronic diseases such as cancer, respiratory diseases, etc. Limited studies have investigated occupational exposure assessments for firefighters; but most have been conducted during training exercises. The fires at the training environment are normally compartment fires and differ from real fire incidents in terms of compartment characteristics, fuel, ventilation profile, etc. The objective of this study is to determine firefighters' exposures to toxic compounds during real fire incidents. Twenty eight firefighters from four fire stations across Ottawa, Canada voluntarily participated and samples were collected from 29 fire incidents in this study (February-October, 2015). Ambient air was collected by the participants to determine the concentration of 16 PAHs (listed in the USEPA) and selected metals (cadmium, Cd; antimony, Sb; and lead, Pb) at real fire incidents. Wipe samples (skin, personal protective equipment (PPE), and personal clothing) were also collected from the firefighters pre and post-fire call to analyze for total PAHs and metal deposition. Concentrations during live fire events were: total PAHs: 5.2 to 28,600 µg/m^3, Cd: 0 to 6.8 µg/m^3, Sb: 0.02 to 170 µg/m^3, and Pb: 0 to 665 µg/m^3. Total PAHs and lead concentration exceeded occupational exposure limit for short duration in some incidents. Firefighters use a self-containing breathing apparatus (SCBA) during firefighting which is supposed to provide them a protection factor of 10,000. Thus, firefighters using SCBA should not be exposed to such high concentrations. However, failure to use SCBA, premature removal of SCBA, or poor-fit can lead to exposure to high concentrations of PAHs and metals. Deposition on skin, PPE, and personal clothing was significantly different between pre and post-fire for total PAHs, Sb, and Pb. Significant deposition on personal clothing suggested that chemicals can leak through the PPE resulting in whole body exposure to chemicals. Handling of equipment during fire and post-fire handling of PPE could also contribute to the deposition. These findings showed that firefighters are exposed to higher level of PAHs and metals during real fire incidents. These information will be used for assessing the hazardous risks associated with exposure to these chemicals during real fire incidents and developing procedures and policies that will effectively reduce exposures.

RP080 Determining the potential bioavailability of copper in apple snails to an avian predator using a simulated digestion method

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As part of the Comprehensive Everglades Restoration Plan, former agricultural lands in South Florida are being acquired for conversion into stormwater treatment areas (STAs) designed to reduce nutrient flows into Everglades National Park. A large proportion of these lands are former citrus groves that were subject to copper-based fungicide applications for decades. As a former citrus grove is inundated with water during the conversion to an STA, colonization by the native Florida apple snail (Pomacea paludosa) and exotic apple snails is likely. Recent research has shown that Florida apple snails grown in systems with elevated sediment copper concentrations have the ability to accumulate large amounts of copper in their soft tissues. In order to accurately assess the risk that copper-laden apple snails pose to the federally endangered Everglade snail kite (Rostrhamus sociabilis), a species that forages exclusively on apple snails in South Florida, determining the fraction of copper in apple snail tissue that is potentially bioavailable to an avian predator is necessary. Florida apple snails grown in copper-impacted soils from citrus groves and field collected exotic apple snails from agricultural canals were processed using methods to simulate avian digestion. The fraction of soluble copper, which is assumed to be potentially bioavailable, and the fraction of insoluble copper in apple snail tissues were isolated and quantified. Preliminary results suggest that >70% of an apple snail's copper body burden is soluble in a digestive environment and potentially available to an avian predator.

RP081 Vulnerability and risks to floods and sewage spill in coastal cities

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The socio-environmental vulnerability is defined as "the deterioration in a particular locality of the physical, economic and social conditions (particularly those related to the urban context) which can lead to the breakdown of the quality of life of its inhabitants." The threats they can arise from a combination of physical and social processes. Thus, human vulnerability integrates many environmental problems. Some are natural phenomena threats, including extreme events such as floods, droughts, fires, storms, tsunamis, landslides land, volcanic eruptions, etc. Coastal cities suffer the river floods cause are prone to flooding including overflowing of water bodies, often contaminated, representing a risk of exposure of inhabitants to contaminants. In this work the risks of exposure associated with flood events in coastal cities of the Río de la Plata river contamination is addressed. Quilmes, Berazategui, Ensenada-Berisso, Magdalena and Punta Indio coastal cities were selected. Urbanized areas with extremely vulnerable from NBI indicators (structural poverty) and exposure to the threat of flooding were identified in each city. Some of the variables surveyed were: level of settlements, population deficit or no access to drinking water, housing facilities, infrastructure in neighborhoods (eg presence or absence of paved roads, power lines.). Preliminary analysis of the sites seen with respect to the deficit or no access to drinking water the percentage of the population is, in the party of Quilmes 23%, in Berazategui 25%, in Magdalena 46% and in Ensenada 11%. As a result of information obtained risk maps were prepared identifying risk and vulnerable areas to flood events and pollutants in sewage overflows areas.

RP082 Increasing algal productivity using polycultures

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Algae holds much promise as a potential feedstock for biofuels, but large-scale algal production has not yet demonstrated the performance required for the economical production of biofuels. To date, the biofuel industry has focused on algal monocultures- single species of algae that have high growth rates and lipid accumulation potentials when cultured under controlled laboratory conditions. However, scaling up monocultural production has remained a significant challenge because monocultures are extremely susceptible to pests and disease. Further, nutrient and water supplies will become limiting as we scale up production unless new approaches are developed to mitigate these resource constraints. The use of wastewater resources that are rich in nitrogen and phosphorus to supplement a portion, or completely replace, traditional fertilizer and water requirements may help significantly reduce algal production costs and save the precious freshwater resources. We performed a laboratory experiment by growing 16 algal species communities (5- monoculture, 10- two species culture and 1 five species culture) in four separate nutrients media. Three of the media were made up separately with nitrate, ammonium and urea as a nitrogen source and another heterogeneous medium that simulates recycled (not fresh or potable) water was created by equal amount of nitrate, ammonium and urea, keeping nitrogen concentration constant. This study demonstrates increased productivity using multi-species algal assemblages, or polycultures grown in heterogeneous growth media. Nutrient uptake efficiency is highly correlated with biomass production. We suggest that cultivating and managing algal polycultures in wastewater may be a more sustainable and cost-effective solution for the biofuel industry than the current practice of monoculture production.

Soil and Water Contaminants: Evaluation, Biomonitoring and Abatement Bioindicators for Effective Management

RP083 Post-impact assessment of an oil spill site in Agaye, Lagos State, Nigeria, and role of metallothioneins in L. violaceous tolerance to oil spill

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Agaye has experienced frequent spills of premium motor spirit due to pipeline vandalization. These contaminated the water sources and farmlands, and with the attendant inferno, destroyed the soil biota. This study determined the pH, Total Petroleum Hydrocarbon (TPH) and the heavy metals (Cd, Cu, Ni, Zn and Pb) levels of the soil and in the earthworm species. It also investigated the abundance of earthworms and presence of metallothioneins (MTs) in the most tolerant earthworm species. Soil samples were collected along transect of spill. Earthworms were handpicked from topsoil. Soil samples were analyzed using standard APHA methods. Heavy metal concentrations in tissue of the two most abundant earthworm species were determined using atomic absorption spectrophotometry. Presence of MTs gene code in the most abundant species was also carried out using standard molecular methods. Mean pH ranged from 5.3±0.04 to 6.5±0.02. Soil TPH, cadmium and copper reduced significantly during the last 11 months. Only soil TPH level was significantly higher than control soil. Lybiodrilus violaceous, Dichogaster modigliani Ephyriodrilus afroccidentalis and Heliodrilus lagosensis were encountered in both sites. The abundance of earthworm 500m away (204 earthworms/m^2) was significantly higher than within the epicenter (45 earthworms/m^2) in the first 12 months but not significantly different in the last 11 months. Significant levels of Pb, Cd, Cu, Zn and Mn in L. violaceous was recorded but Ni was not detected. Only zn and Cd were detected in D. modigliani. The increase in number and species of earthworm in the last 11 months indicated possible remediation of the environment. The high concentrations of heavy metals in the earthworms suggest possible roles in bioaccumulation. The presence of metallothiens genes could elucidate the mechanism of tolerance of L. violaceous.

RP084 Remediation of Ni Toxicity by Liming – Field Validation

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Toxicity of metals is dependent on soil chemistry, especially pH. Liming of field soils contaminated with Ni in Port Colborne ON was studied as a remediation technique. Changes in pH not only affect bioavailability of metals but also soil nutrients and may cause adverse effects to plants. Treatments of 88t/ha of calcitic or dolomitic lime as well as positive control with no lime (mechanical equipment only) and a negative control with no treatment were applied to a field in Port Colborne early in 2015. The soil was sampled before and after lime application, and soybeans planted in July 2015. Soybean was harvested in October and yield was calculated. Soil pH, pseudo-total Ni, plant-available Ni (using $CaCl_2$) were measured on samples collected before and after liming. It was found that both types of lime increased soil pH, though calcitic lime resulted in a greater increase than dolomitic. A decrease in pseudo-total Ni was seen following liming, though this could be explained by the dilution effect of adding 88t/ha of lime to the top 5 cm of soil. Plant-available Ni was reduced by both types of lime; however calicitic had a greater effect than dolomitic. It was found that there is a very predictable relationship between soil pH and plant available Ni, which does not depend on the way the pH was increased. Soybean yield was significantly lower than that observed for Ontario due to late seed planting. The greatest yield was seen on the negative control; due to large error for that treatment the negative control yield was statistically greater than only the calcitic treatment and not the dolomitic. Higher yield was seen in the dolomitic treatment than calcitic, which may be due to calcitic pH being too high for optimal soybean production.

RP085 Altered calcium metabolism, blood lead levels and intelligence quotient scores in school children in Ibadan, Nigeria

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Lead (Pb), a heavy ubiquitous metal, is one of the oldest and most studied environmental contaminant known. Exposure to this metal and its associated toxicity, remains a continuous problem especially in developing countries and children are mostly at risk. Childhood environmental lead (Pb) exposure has been linked to intelligence quotient (IQ). Lead neurotoxicity associated with cholinergic neurotransmission may be induced by altered calcium(Ca) homeostasis. The interplay between childhood environmental lead exposure and calcium metabolism as well as intelligent quotient has not been sufficiently explored in environment with prevailing nutritional deficits and related events. This study was therefore, conducted to address this gap in knowledge. Three hundred and nine healthy school children aged 6-12 years were recruited and divided into 2 groups; 169 with elevated blood lead level (EBLL) and 140 with BLL within acceptable limit as controls. Both groups were certified clinically free by a Paediatric Neurologist. Intelligence quotient was determined using Raven's Standard Progressive matrices. Red cell cholinesterase (ChE) activity and serum vitamin D level were determined using HPLC, blood lead was assessed with AAS while total Ca, phosphate and magnesium (Mg) were determined using standard spectrophotometric methods and ionized calcium levels were computed using standard formular. The ChE activity and vitamin D levels were significantly higher in children with EBLL compared with control ($P< 0.05$) while IQ, levels of total Ca and ionized Ca were significantly lower in children with EBLL compared with control ($P< 0.05$). The levels of phosphate and Mg were similar in both groups ($P>0.05$). Blood Pb demonstrated significant positive correlation with ChE activity and vitamin D. In contrast, non-significant negative associations were observed between blood Pb and IQ score, ionized Ca, total Ca, phosphate and Mg levels in children with EBLL. Environmental childhood Pb exposure was associated with alteration in Ca metabolism which manifested as a possible perturbation in Ca regulated events and may have implications for intelligence quotient. Dietary strategies based on calcium may be recommended for children at risk of Pb exposure to reverse neurochemical correlates. Our findings might have important implications for environmental policies, especially those designed to protect children's health and prevent factors that are operative early in life.

RP086 Elevation in estrogen level as a marker in chronic exposure of environmental PAHs

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This paper is a part of an on-going investigation of the long standing effect several crude oil spills in Eket, Akwa Ibom State, Nigeria, in which both a vast land area and Qua Ibo River were greatly polluted with petroleum hydrocarbons. The monitoring programme includes screening of PAHs in the environmental media, evaluation of bioaccumulation of PAHs in aquatic organisms exemplified with tilapia, and determination of in vitro endocrine disruptors in the women population of reproductive age. Based on a total of 88,635 women in Eket (Nigeria population census figures of 2006) and using a simplified sample size formula, a total of 186 reproductive age women were selected for blood sampling. The assay for endocrine disruptors involved determination of free E2 and SHBG concentrations in the selected women, after which the ratio of SHBG to E2 was determined. Blood serum samples were prepared from the blood collected and the assay carried out using AccudiagTM Estradiol and SHBG ELISA kits. The result shows that 47 individuals of the 186 women of reproductive age used for the analysis have elevated estrogen level beyond the recommended reference range of 15-60pg/ml, and this accounted for approximately 25% of 186 women. Also results showed variable concentrations of PAHs in water, sediment and in fish samples. Most apprehensive is the elevated concentrations of some PAHs beyond permissible limit in the fish and this calls for concern with respect to public health and safety. The sixteen priority PAHs listed by the United State Environmental Protection Agency as carcinogenic were detected. Comparing this results with our control reference community where there was relatively very low level of environmental PAHs, one can infer that the women community of Eket are more predisposed to breast cancer due to high level of PAHs in their environment, probably the result of incessant oil spill (pollution) incidents within their community. This investigation recommends that Eket women within reproductive age should receive anti-estrogen therapy intervention to reduce the risk of breast cancer. <br style="font-size: 12.0pt; font-family: 'Times New Roman','serif'; mso-fareast-font-family: Calibri; mso-ansi-language: EN-US; mso-fareast-language: EN-US; mso-bidi-language: AR-SA;" />

RP087 Pharmaceutical residues in surface water around Livestock Agricultural farms

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The health benefits, and the derivable economic advantages of the administration of pharmaceuticals and pharmaceutical products in livestock agricultural farms, have led to an increase in the dependence and use of these products. Substantial amount of pharmaceuticals pass into the environment either in the form in which they are administered, or as metabolites via animal excretion and wash/wastewater release from domestic, industry and farm sources into nearby surface water. In this study, a method which allows for the simultaneous determination of the residues of Tetracycline, 17β-estradiol, acetaminophen, and ivermectin in aqueous medium was developed using High Performance Liquid Chromatography coupled to a Diode Array Detector(HPLC-DAD). Water samples were collected from some water channels traversing the vicinity of some agricultural farms and wastewater streams in Cape Town environment. The samples were pre-concentrated by Solid Phase Extraction(SPE) using methanol pre-conditioned hydrophilic-lipophilic balance(HLB) cartridges. Residues of the pharmaceuticals were thereafter extracted into methanol. The extracts were thereafter analyzed for 17β-estradiol, tetracycline, acetaminophen and ivermectin using HPLC-DAD. Results showed that the concentrations of Tetracycline (< 0.08-26.74 µg/L), 17β-estradiol(< 0.36–5.92 µg/L), acetaminophen (< 2.80-9.67 µg/L), and ivermectin(< 0.1405-11.65 µg/L) generally varied and site dependent. There was no specific trend pattern to this. Seasonal weather variation, flow pattern, and physico-chemical quality of the water may account for the variations in observed concentrations. Analyte recovery achieved ranged between 76 and 88%, however the HLB solid phase extraction procedure may be manipulated to achieve better recovery. The selectivity and sensitivity of the method were also discussed.

RP088 Phenanthrene abatement from aqueous media using Vitis vinifera leaf litter

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The deterioration in quality of freshwater bodies due to influx of toxic organic contaminants into the systems poses serious health risks to humans and aquaculture. These organic contaminants are capable of causing acute (diaphoresis and dermal irritation) and chronic (cancer and endocrine disruption) diseases. Anthropogenic activities have been reported to be major pathways of pollutants such as PAHs. PAHs have been reported to be ubiquitous and recalcitrant in the environment. This study therefore explored the potential of low cost adsorbents prepared from Vitis vinifera (grape) leaf litter for the remediation of phenanthrene from wastewaters. Adsorbents were prepared by activating raw biomass with H_3PO_4 and $ZnCl_2$ at various biomass/reagent ratios. Activated adsorbents were carbonized at 600 ^{o}C for 1 h under nitrogen stream. The percentage adsorbent yield was varied with activating reagents and values ranged between 41.63-58.40and 44.65-47.08 for H_3PO_4 and $ZnCl_2$ respectively. The moisture content, ash content, crude fibre content, carbon content and sulphur content of the precursor and activated adsorbent were evaluated and discussed. The adsorption of phenanthrene from aqueous media by raw and carbonized adsorbents was investigated as a function of adsorbent dosage, initial adsorbate concentration and contact time. Various adsorption isotherms and kinetic models were used to interprete the mechanisms of PAHs' removal by adsorbents.

RP089 Wastewater remediation: Batch adsorption of ciprofloxacin onto zeolites

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Ciprofloxacin, a second generation of FQ's antibiotics is globally used for the treatment of several bacterial infections in humans and animals. It may reach the aquatic ecosystem from domestic discharge, or from the effluents of drug manufactures. Generally, 30 -90 % of the active ingredients are excreted un-metabolised after injection, due to incomplete metabolism in human and animals. The occurrence of ciprofloxacin in water even in low concentrations, can lead to the emergence of antibiotic resistant bacteria and other adverse consequences. Insufficient removal of antibiotics in conventional wastewater treatments imposes an examination of their removals by alternative methods. Low cost carbon-adsorption method has attracted researcher's interest because of its simplicity. Currently, the use of synthetic zeolites adsorbents are evaluated as appropriate adsorbents due to its low cost and high removal efficiency. In the present work, the adsorption of ciprofloxacin utilising coal fly ash based zeolites as driving force for the remediation of wastewater was investigated, with an intent to achieve the most effective, low cost and environmental friendly adsorbents. Studies on the surface morphology of the zeolites were carried out using SEM and TEM, while the identifications of mineral phases were carried out by using XRD. The physio-chemical properties such as the specific surface area, density, and particle size and lattice vibration were obtained with BET and FTIR respectively, with the adsorption monitored by HPLC-UV/Vis. The effective use of zeolites formulated from fly ash, in the decontamination of wastewaters contributes to the mitigation of coal fly ash (natural by-product) and reduce its environmental impact.

Ideas and Concepts for Dissemination and Communication of Research Findings in Times of Open Science and Science 2.0

RP090 Highlights: An interactive workshop designed to bring social and environmental scientists together to improve wildfire smoke health risk messages

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Scientists are asked to communicate their research for a variety of reasons, including general transparency of science, completing funding/grant applications, or making contributions to the greater body of research supporting public and environmental health. Conveying messages that are accessible, comprehendible and actionable requires an understanding of the audience. Particularly how the audience acquires and uses science-based information. Wildfires are increasing in intensity and frequency, meaning more smoke. Information about pollutants (e.g., PM2.5 and ozone) from wildfire smoke is important to enable protection of cardiovascular and pulmonary health of both healthy and sensitive populations. Low levels of smoke can be intangible and often invisible, resulting in perceived risk that is lower than actual risk. It is therefore critical to improve health risk messaging surrounding wildfire smoke, and provide tools that allow people to make decisions on how to reduce exposures and protect their health. In September 2016, the USEPA will host a workshop designed to investigate understanding, communicating, and managing health risks associated with wildfire smoke exposure. This unique workshop will bring together social and environmental scientists with community and institutional stakeholders to deconstruct this complex issue. Through collaborative engagement activities and interactive presentations workshop participants will develop a shared and multidimensional understanding of the nature of the public health problem associated with smoke exposures. This "understanding" should reflect scientific evidence and community attributes and experiences, as well as information and actions related to risk communication and management appropriate for further scientific evaluation. The expected workshop outcomes are: 1) an understanding of the network and players involved in wildfire smoke health risk communication; 2) an integrated "mind map" of the perspectives around the problem; 3) behavioral maps that illustrate step by step behaviors an individual would take to adopt health protective behavior, and the most influential step to deliver a message; 4) an understanding of what works with current messaging; and 5) integration of insights into a mobile app ("SmokeReady"), to be tested in 2017 using a crowd sourcing method, intended to improve access and delivery of health risk messages. This abstract does not necessarily reflect USEPA policy.

RP091 Oceanbites.org: Engaging Online Audiences with Stories of Oceanographic Research

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In 1876, the HMS Challenger completed the world's first oceanic research expedition, bringing back news that the deep seas were teeming with life, and characterizing the ocean's complex currents and chemistry for the first time. Oceanography has captured the public imagination ever since, but finding out what's going on in current oceanography research can be very difficult for a non-expert. Journal articles are often extremely detailed and full of jargon that can be difficult even for other scientists to parse. Oceanbites.org is a blog written and run by graduate students that provides summaries of recent cutting-edge oceanography studies, written in a style that is engaging for anyone with a high school-level science background. The site was founded in September, 2013 with two goals in mind: to make new research more accessible to the non-expert public, and to provide graduate students with the opportunity to practice honing messages for broad audiences. Currently, more than 20 graduate students from universities around the world contribute regularly to the site, and it has grown to have dedicated followings on social media, with more than 10,000 Twitter followers, and more than 600 Facebook followers. Oceanbites publishes one article per weekday as well as occasional posts on science communication and popular media related to marine sciences. Other features to engage readers include biweekly Spanish translations and theme weeks focused on topics voted for by our readers. In this presentation, Oceanbites' journey so far will be described, as will our future aspirations to engage more undergraduates in producing content for the site by running blog-writing workshops, and our plans to involve our readers in ocean and environment-related citizen science projects.

RP092 Data exploration, Public Transparency and Enhanced Outreach through the use of Data Visualization Tools: PAHs in Ambient Air in Minnesota

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This interagency project aimed to characterize air concentrations of vapor and particle-phase PAHs spatially and temporally in an inner city community and a rural site, to investigate sources, estimate Benzo[a]pyrene equivalent cancer potency, and compare measured and modeled concentrations. Three existing ambient air monitoring sites were chosen for active air samplers. Siting of 20 cylindrical passive air samplers (Wania et al 2003, Schrlau et al. 2011) was informed by modeled concentrations, community input, potential Environmental Justice areas, and proximity to known pollutant sources and sensitive receptors. Air sampling began in June 2013 and continued for 2 years. Active air samples were collected for 72 hours every 12 days. Passive samplers were deployed continuously for a sampling duration of 3 months. PAHs were extracted (size-fractionated XAD-4, Quartz Microfibre filters) using a Dionex ASE-350, separated using a Varian Select PAH, 30m x 0.25mm (0.15µm film thickness) analytical column, and analyzed by Agilent 5977 GC/MS. The PAH analyte list was selected to maximize the number of PAHs analyzed with cancer potency information, and the number of PAHs that inform source apportionment. As a governmental agency, it is important for our work to be transparent to our very interested and environmentally educated general public. We developed an interactive data tool in order to release our data in a format that allows interested users to interact directly with the data and potentially answer some of their own questions. The interactive data visualization tool utilizes Tableau Software © and is embedded in the project website. The tool allows users to chart data by sampler type (passive, gas phase, particle phase, total concentrations), and compare summarized results to inhalation health benchmarks. The results are also available in a mapped format, in which PAHs are selectable and concentrations that are summarized for the entire study and by season are displayed. These types of data visualization tools allow presenters to provide a planned tour of the data, and then open up the conversation to audience focused questions and interests. The data visualization tool will be presented in this format, with a planned talk through the project and data, and then opened up for audience based question and answers.

RP093 MOSAIC: A web interface with modelling and statistical tools for ecotoxicology

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MOSAIC stands for "MOdeling and StAtistical tools for ecotoxicology". In ecotoxicology, bioassays are standardly conducted in order to measure acute or chronic effects of potentially toxic substances on reproduction, growth and/or survival of living animals. MOSAIC has been designed as a user-friendly web-interface to disseminate the mathematical and statistical modelling of standard bioassay data. Its simple use makes MOSAIC a turnkey decision-making tool for the community of ecotoxicologists, both from academia and industry, and regulators. Without wasting time on extensive mathematical and statistical technicalities, the users are given the most advanced and innovative methods for a robust quantitative environmental risk assessment. MOSAIC is freely available at http://pbil.univ-lyon1.fr/software/mosaic/. Today, MOSAIC offers three operational tools : (i) MOSAIC_SSD, a tool dedicated to the species sensitivity distribution (SSD) approach aiming at defining safe levels for toxic compounds in an ecosystem through the calculation of the so-called hazardous concentration for p% of the species (HCp), even when the toxicity values are censored; (ii) MOSAIC_repro, which provides users with a complete statistical analysis of bioassay reproduction data simultaneously accounting for mortality all along the bioassay. Concentration-response models are fitted within a Bayesian framework to provide ECx estimates; (iii) MOSAIC_surv, which provides users with a complete statistical analysis of bioassay survival data. This presentation is an overview of the current MOSAIC features based on illustrative examples as provided within the web interface, as well as of the surrounding MOSAIC facilities, like the R code freely offered to the users to ensure transparency and reproducibility of analyses and a "pdf" button to easily export and save results. Future extensions of MOSAIC will be also presented in particular the possibility to comfortably fit TK-TD models.

Alternative Assessments Best Practices for Safer Chemistry

RP094 Update and Redesign of USEPA Office of Pollution Prevention and Toxics Predictive Tools EPISuite™ and ECOSAR

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Under the Toxic Substances Control Act, the Office of Pollution Prevention and Toxics (OPPT) regulates both new and existing industrial chemicals. For new chemicals, OPPT makes a regulatory decision on each chemical, often with limited information on the specific chemical or chemical class. Given the large number of chemicals submitted each year and the fact that ~65 percent of them are submitted with limited data, OPPT relies on computational tools to predict over 150 attributes in order to make rapid decisions regarding hazards and risks of these materials. For over 25 years, the gold standard for the prediction of physical/chemical properties, environmental fate parameters, and ecotoxicity attributes for the assessment of new chemical substances has been the EPISuite™ and Ecological Structure Activity Relationship (ECOSAR) programs. A new Java-based, object-oriented, web version of EPISuite is under development. Improvements are focusing on an enhanced user experience as well as value-added data displays. Based on a web-services architecture, EPSuite™ estimates will also be accessible to remote servers. A new object-oriented version of ECOSAR is nearly completed and the latest update includes comments from an extensive beta test. The new features incorporated into these tools will be the focus of this presentation. In addition to the software improvements, updates to the QSAR models in these tools and models will be presented. For example, ECOSAR Version 2 contains new algorithms for surfactants, dyes, and polymers based on information and knowledge gained from the new chemicals program in OPPT. The QSARs are for acute and chronic toxicity endpoints for fish, aquatic invertebrates, and algae (surrogate species used in standard EPA new chemicals program aquatic toxicity profiles). Updates to EPISuite™ include new algorithms that enhance the predictive accuracy of highly fluorinated and silicon-containing compounds. The EPISuite™ update also include a new method for determining if estimates lie within the domain of applicability of any given model, which leverages the capabilities of OPPT's Analog Identification Methodology (AIM). The views expressed in this abstract are those of the authors and do not represent Agency policy or endorsement.

RP095 A QSAR to Predict Anaerobic Biodegradability of Hydrocarbon Mixtures

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The biodegradability of chemicals speaks to their persistence, their risk, and subsequently their regulation. Most biodegradability testing and models focus on aerobic fate, but anaerobic conditions play a major role, particularly in wastewater treatment plants, contaminated sediments, and certain disposal practices. One relevant example is for the disposal of cuttings generated during drilling of oil & gas wells in the Gulf of Mexico. Part of the criteria for offshore disposal of the drill cuttings is that the drilling fluids used must pass an anaerobic biodegradability test, the Modified ISO 11734:1995 EPA Method 1647. In this method, the drilling fluid is mixed in a closed bottle with sediment from the Gulf of Mexico, and gas production indicative of methanogenic biodegradation is monitored over 275 days. Drilling fluids "pass" the anaerobic biodegradation test if they biodegrade at the same rate, or faster than a C1618 internal olefin standard. Producers of drilling fluids must test their drilling fluids on a yearly basis, which means that over the past 15 years, a large amount of anaerobic testing data has been generated. In 2014-2015, Shell Oil Company (USA) worked with a contractor to create an anaerobic biodegradation model based on 176 samples that underwent the ISO11734 test. This model addresses 2 unique variables: anaerobic biodegradation of hydrocarbons and working with defined mixtures. The model developed was able to account for the multi-component nature of the drilling fluids, and good results were obtained when predictions were made for mixtures that were within the applicability domain of the model, as defined by both the quantity and type of hydrocarbons present. The validation exercise (performed on data not used in model development) predicted 71% true positives, 92% true negatives, and had 79% concordance when pass–fail criteria were applied. While the model is not complete and suffers from limitations in the training data set, this data and approach can be adapted and developed for other types of hydrocarbons and other anaerobic conditions. The model can be used as a screening tool, to optimize anaerobic biodegradability of products, or to estimate performance of anaerobic biodegradation systems (e.g., anaerobic bioreactors, sediments, etc.). This presentation will discuss the data and design of this QSAR and the need for this type of model, especially from an industrial perspective.

RP096 Update and Reevaluation of the EPISuite™ MITI BIOWIN 5 and 6 Biodegradation Models

M. Kawa, SRC Inc. / Environmental Health Analysis; D. Lynch, EPA / OPPT RAD; W. Lee, EPA / OPPT; K. Nakayama, NITE; W. Meylan, SRC, Inc.; J.L. Tunkel, SRC, Inc. / Environmental Health Analysis

Under the Toxic Substances Control Act, the Office of Pollution Prevention and Toxics (OPPT) regulates both new and existing industrial chemicals. For new chemicals, OPPT makes a regulatory decision on each chemical, often with limited information on the specific chemical or chemical class. Given the large number of chemicals submitted each year and the fact that ~65 percent of them are submitted with limited data, OPPT relies on computational tools to predict over 150 attributes in order to make rapid decisions regarding hazards and risks of these materials. For over 25 years, the gold standard for the prediction of physical/chemical properties and environmental fate parameters has been the EPISuite™ program. The EPISuite™ models, BIOWIN 5 and 6, are predictive models for assessing a compound's potential for rapid biodegradation in the Ministry of International Trade and Industry (MITI-I) test originally derived on 884 chemicals, with 385 "readily degradable" chemicals and 499 "not readily biodegradable" chemicals. Re-evaluation of these models was performed using data from Japan's National Institute of Technology and Evaluation (NITE). The revised dataset contains 1,372 chemicals with well documented biodegradation data obtained according to MITI-I test guidelines. The current works adds over 700 chemicals to the training and validations sets. The BIOWIN 5 and 6 domain is for discrete organic chemicals. Mixtures, reaction products, polymers and chemicals that undergo rapid hydrolysis, or volatilization were not included in the re-evaluation; a process that was facilitated using the ChemACE clustering tool. Potential outliers and studies requiring a more detailed examination were also identified through cluster review. Evaluation of the results from different methods of analysis, including TOC, GC and HPLC, identified chemicals that may have degradation products with slower ultimate removal (low TOC and high GC or HPLC). Multiple linear and nonlinear regressions using new and existing BIOWIN fragments and descriptors were assessed so that the structural fragment library could be re-evaluated to improve BIOWIN 5 and 6 model performance.

RP097 The Quest for Safer Consumer Products through Alternatives Analysis

H. Muniz, California Dept of Toxic Substances Control / Safer Consumer Products

The Safer Consumer Products regulations, called for in California's Green Chemistry Initiative, created a practical, meaningful and legally defensible approach to identifying and moving toward safer consumer products. Using a science-based process during a product's design phase, the regulations require the identification of toxic ingredients and the analysis of alternatives to safer ingredients. Based on the results of the analysis a number of steps can be taken, including removal of the toxic ingredients, posting product information for consumers, further research and development and safety measures. Ms. Muniz, a principal drafter of the Safer Consumer Products Regulations, will provide an overview of the timelines and duty to comply; the chemical and priority product prioritization process; the petition process; the alternatives analysis process and the regulatory responses.

RP098 Advancing Alternatives Assessments: Ongoing Efforts of OECD's Ad Hoc Group on Substitution of Harmful Chemicals

E. Connor, Abt Associates / Environment Resources Division; K.M. Hart, USEPA

This presentation will highlight recent efforts of OECD's Ad Hoc Group on Substitution of Harmful Chemicals to advance the practice and understanding of alternatives assessments. The OECD's 49th Joint Meeting of the Chemicals Committee and the Working Party on Chemicals, Pesticides, and Biotechnology established the Ad Hoc Group with the goal of furthering tools and approaches to support decision making for substitution of chemicals of concern. This presentation will highlight two ongoing projects of the Ad Hoc Group -- updates and enhancements to the OECD Substitution and Alternatives Assessment Toolbox (SAAToolbox), and the capture and analysis of alternatives assessment case studies. The case study project aims to summarize real world cases of chemical substitution in a systematic way to facilitate the analysis of best practices, lessons learned, and benefits of chemical substitution. The resulting case study repository will be integrated into the SAAToolbox. For each project, the approach and results to date will be presented. As the demand for alternatives assessment approaches continues to grow (e.g., in response to the European Union's REACH regulation and California's Safer Consumer Products Regulations), the SAAToolbox and other project work of the Ad Hoc Group serve as important resources to assist practitioners in identifying and implementing appropriate methods and tools for conducting them.

RP099 IC2 Level 1 Alternative Assessment for Refrigerants in Domestic Refrigerators

D. Skall, T. Lewandowski, K. Reynolds Reid, J.M. Cohen, Gradient

Efforts to address global climate change have included phasing out the use of hydrofluorocarbons as refrigerants and propellants in many products. R-134a, the main refrigerant used in US household refrigerators, has a global warming potential (GWP) of 1,300, (i.e., 1,300 times that of carbon dioxide [CO_2]). Given that millions of refrigerators are purchased in the US each year, switching to a low-GWP refrigerant could substantially impact US greenhouse gas emissions. However, changing the main refrigerant in US refrigerators will likely require trade-offs, and Alternative Assessment (AA) can be used to evaluate the possible trade-offs of using various chemical substitutes for R-134a. To explore how an AA for residential refrigeration might work, we conducted a Level 1 AA according to the Interstate Chemicals Clearinghouse (IC2) Alternatives Assessment Guide. This first-phase analysis involves using readily available information in order to determine whether a more in-depth evaluation is worthwhile. We compared five possible alternatives to R-134a: propane, isobutane, ammonia, CO_2, and tetrafluoropropene (R-1234yf). We evaluated the hazards of these chemicals, which is a key part of an AA, using GreenScreen® for Safer Chemicals, an open source scoring system that is increasingly used in product stewardship programs. A simultaneous decision framework was used to evaluate the different IC2 modules (e.g., performance, hazard, exposure). CO_2 was screened out at the initial stage due to the lack of data concerning its use in domestic refrigeration. We found that when the standard GreenScreen benchmark, which does not include consideration of GWP, was used to evaluate chemical hazards, only propane was found to be preferable to R-134a. Including GWP as a variable in benchmarking resulted in all four alternatives being identified as preferable to R-134a. When GreenScreen hazard scores were evaluated in a more in-depth manner (i.e., comparing alternatives across individual health endpoints), propane, isobutane, and R-1234yf were all identified as preferable to R-134a; ammonia was rejected due to substantial human and ecological toxicity. Data gaps and questions that should be addressed in a more detailed Stage 2 AA will also be discussed.

RP100 Preparing for the new TSCA: how the TSCA Work Plan and EPA Safer Choice Standard can help

J. Nusz, E. Freeman, Exponent Inc; J.P. Staveley, Exponent

The TSCA Work Plan and the EPA's Safer Choice Standard both provide useful frameworks for evaluating the relative risk profiles of chemicals and products. Each of these frameworks evaluate chemical risks taking into account both the inherent hazard of the chemical and the potential for exposure resulting from a particular use. Companies can use these frameworks and criteria provided therein to effectively triage chemical/product inventories to identify chemical uses of highest and lowest relative risk. This type of assessment is useful for planning the allocation of financial resources and timing needed for management of inventory. For example, chemical uses that meet the Safer Choice Standard hazard criteria are likely to be of lower concern to EPA when prioritizing chemicals for review under TSCA. Further, they may be good candidates for submittal to EPA's Safer Choice Program. In contrast, chemical uses identified as having high relative risk through the TSCA Work Plan framework, or those with extensive data gaps, may require further investigation and allocation of resources. It is important to note, that while TSCA reform may result in changes such as EPA authorization for requesting generation of new data to fill data gaps, changes to the evaluation framework for prioritization of chemicals provided in the TSCA Work Plan Chemicals: Methods Document are not expected. We will compare and contrast the data required under both frameworks for the evaluation of ecological and human health hazards, potential for exposure, and risk.

RP101 Assessment of Soil Microbial Community Compositional and Functional Shift in Biodiesel vs. Petrodiesel Contaminated Soil

M. Dong, Texas Tech Univ / Biological Science; D.L. Carr, Texas Tech Univ / Biological Sciences

Soil microorganisms participate in many critical ecosystem processes, including nutrient mineralization, formation and enhancement of soil structure. Their activities and compositional changes due to contaminants could be indicators of soil health. Biodiesel is considered as a viable substitute for petroleum diesel primarily because it performs well, and it is assumed safe, readily biodegradable, and environmentally friendly. This study compared the effects of petrodiesel and three types of biodiesel on soil microbial community. Contaminated soil samples were tracked for 180 days to determine the degradation rate of the contaminants and their effects on the composition and function of soil microbial community. GC-FID was used to determine the degradation rate of contaminants while commercial Biolog EcoPlates™ were used to test the microbial community function based on carbon usage. Soil microbial composition were addressed by 16s rRNA gene sequencing. Results suggest that biodiesels are not statistically different from petroleum diesel in terms of their adverse impacts on soil microbial function. In addition, site restoration potential was evaluated by seed germination and plant growth in diesel and biodiesel contaminated soils for over 35 days. ANOVA results show all groups have similar plant germination rates. Petrodiesel treatments exhibit significant decrease in total plant length. One biodiesel, castor methyl ester, has a statistically similar yield of plant dry weight compared with plants grown in the untreated soil control. In conclusion, our results suggest that the chemical structure of biodiesels might determine whether they are environmentally benign, and that they should not be automatically considered as harmless substitutes for diesel.

RP102 Incorporation of Terrestrial Toxicity Hazard Endpoints into the GreenScreen® for Safer Chemicals Evaluations of Three Neonicotinic Pesticides

J. Rutkiewicz, ToxServices; M. Whittaker, ToxServices LLC; J. Sass, Natural Resources Defense Council

The GreenScreen® for Safer Chemicals is a comparative hazard assessment tool designed to evaluate chemicals across 18 human and environmental health hazards. The methodology is publically available and involves assigning hazard scores to each endpoint following a review of authoritative lists and toxicity data, as well as an overall Benchmark™ score, which is a high-level indicator of hazard based on the combination of hazard scores for the individual endpoints. GreenScreen has been used by industry and advocacy groups to evaluate and make decisions regarding chemical use in a variety of product types, is the hazard assessment platform for a number of standards and ecolabels, and is a preferred hazard assessment method for alternatives assessments under several state programs. While the standard GreenScreen ecotoxocity evaluation includes acute and chronic aquatic toxicity, GreenScreen guidance also states that when relevant, additional ecotoxicity endpoints such as avian or bee toxicity may be incorporated using the EPA's Design for the Environment (DfE) Program Alternatives Assessment Criteria for Hazard Evaluation. At the request of the Natural Resources Defense Council, ToxServices incorporated terrestrial toxicity data into GreenScreen assessments of three widely used neonicotinic pesticides, thiamethoxam, imidacloprid, and clothianidin, that are known to be highly hazardous to terrestrial invertebrates such as bees. All three pesticides were assigned Benchmark scores of 1 (Avoid-Chemical of High Concern) due to their very high persistence in combination with hazards for ecotoxicity (clothianidin and thiamethoxam) or ecotoxicity and neurotoxicity (imidacloprid). All three pesticides received the highest hazard scores for acute aquatic toxicity and acute foliar invertebrate (i.e. bee) toxicity, but only clothianidin and imidacloprid received the highest scores for chronic aquatic toxicity. Clothianidin and imidacloprid received moderate and high scores for acute terrestrial vertebrate (i.e. bird) toxicity. Therefore, evaluation of terrestrial toxicity endpoints illustrated differences in the overall ecotoxicity profile that are not apparent in an evaluation of only the standard GreenScreen endpoints. Given the application of GreenScreen for comparative hazard and alternatives assessments, incorporation of terrestrial toxicity produces a more robust hazard characterization for informed decision making.

Use of Modeling Tools to Determine Environmental Concentrations from Environmental Passive Samplers

RP103 Ambient Air Monitoring for an Expanded List of PAHs in an Urban and Rural Area Using Passive and Active Methodologies

K. Ellickson, Minnesota Pollution Control Agency / Environmental Analysis and Outcomes; M. Krause, Minnesota Dept of Health / Public Health Laboratory Organic Chemistry; C. McMahon, Minnesota Pollution Control Agency / Environmental Analysis and Outcomes Division; C. Herbrandson, Minnesota Dept of Health / Environmental Health retired; C. Schmitt, Minnesota Dept of Health / Public Health Laboratory Organic Chemistry; C. Lippert, Mille Lacs Band of Ojibwe Dept of Natural Resources and Environment / Air Quality; G. Pratt, Univ of Minnesota / School of Public Health Division of Environmental Health

This interagency project aimed to characterize air concentrations of vapor and particle-phase PAHs in an inner city and a rural setting, to investigate sources, estimate Benzo[a]pyrene equivalent cancer potency, and compare measured and modeled concentrations. Three existing ambient air monitoring sites were chosen for active air samplers. Siting of 20 cylindrical passive air samplers (Wania et al 2003, Schrlau et al. 2011) was determined by modeled concentrations, community input, potential Environmental Justice areas, and proximity to known pollutant sources and sensitive receptors. Air sampling began in June 2013, the beginning of meteorological summer. Active samples were collected for 72 hours every 12 days, and passive samplers were deployed continuously for a sampling duration of 3 months, over a total sampling period of 2 years. PAHs were extracted (size-fractionated XAD-4, Quartz Microfibre filters) using a Dionex ASE-350, separated using a Varian Select PAH, 30m x 0.25mm (0.15µm film thickness) analytical column, and analyzed by Agilent 5977 GC/MS. The PAH analyte list was selected to maximize inhalation risk assessment and source apportionment information. The passive sampler mass loading was calculated based on the seasonally averaged total (gas plus particle) active sampler results. Annual and whole study upper 95th

confidence limits of the arithmetic mean of air concentrations were calculated without detection limit substitution and compared to Benzo[a]pyrene potency equivalence to assess potential inhalation human health risks. Benzo[c]fluorene was the most potent c-PAH, but requires analysis of a mass interferent Dibenzothiophene sulfone. Several localized events were observed in the PAH concentration data, helping to identify PAH profiles from these sources. Gas-particle partitioning was a function of temperature, sub-cooled vapor pressure, and octanol-air partitioning coefficient, in keeping with previous studies. Retene, a wood smoke marker, is somewhat anomalous because it's vapor pressure is high compared to other PAHs of similar molecular weight. The individual PAHs, and cumulative potency do not exceed human health risk guidelines. Source apportionment work underway will provide source contribution estimates that can be used to inform agency priorities.

RP104 Benzo[c]fluorene Potency in Ambient Air and Dibenzothiophene Sulfone as a Mass Interferent and Potential Gas Phase Diesel Marker

K. Ellickson, Minnesota Pollution Control Agency / Environmental Analysis and Outcomes; M. Krause, Minnesota Dept of Health / Public Health Laboratory Organic Chemistry; C. McMahon, Minnesota Pollution Control Agency / Environmental Analysis and Outcomes Division; C. Herbrandson, Minnesota Dept of Health / Environmental Health retired; C. Schmitt, Minnesota Dept of Health / Public Health Laboratory Organic Chemistry; C. Lippert, Mille Lacs Band of Ojibwe Dept of Natural Resources and Environment / Air Quality; G. Pratt, Univ of Minnesota / School of Public Health Division of Environmental Health

Cancer potency may not be fully assessed for airborne c-PAHs if air monitoring is limited to the EPA 16 priority PAHs. An expanded list of PAHs was measured in ambient air using active and passive sampling in an urban and rural setting. Air sampling began in June 2013, the beginning of meteorological summer. Active samples were collected for 72 hours every 12 days, and passive samplers were deployed continuously for a sampling duration of 3 months, over a total sampling period of 2 years. PAHs were extracted (size-fractionated XAD-4, Quartz Microfibre filters) using a Dionex ASE-350, separated using a Varian Select PAH, 30m x 0.25mm (0.15µm film thickness) analytical column, and analyzed by Agilent 5977 GC/MS. In the urban area, passive samplers were sited based on modeled results, known potential Environmental Justice areas, and community input. Air concentrations were summarized by study year, and for the entire study for cancer potency comparisons. Upper 95% confidence limits of the annual arithmetic mean were calculated without detection limit substitution for comparison to estimated inhalation health benchmarks based on Benzo[a]pyrene cancer potency equivalents. Benzo[c]fluorene had the highest BaP equivalent potency of any other PAH, and therefore drove the inhalation cancer potency for the air mixture. However, Benzo[c]fluorene has a mass interferent of Dibenzothiophene sulfone (DBTS). DBTS is an intermediate in the desulfurization process of diesel fuel from Dibenzothiophene (DBT). Dibenzothiophene is used as a model sulfur-containing diesel fuel component, as the sulfur is less reactive because it is stabilized by the aromatic structure of DBT. Energy is required for the desulfurization of DBT, generally, an oxidant, chemical catalyst, higher than ambient temperature heat or biological reactions with the sulfur in DBT. There is potential, therefore, that DBTS could be monitored as a gas phase diesel marker. It is also possible that DBTS is a sampling artifact created on the XAD-4 surface. A multiple measures model was conducted for DBT/DBTS ratios, proximity to major roadways, comparisons with ambient oxidant concentrations (ozone), ambient temperatures, home heating days, and winter vs summer diesel fuel blend seasons. The statistical analysis was different for passive and active samplers. Our preliminary results are suggestive of a gas phase diesel marker that is detectable using both active and passive samplers.

RP105 Spatial variations of porewater and bulk sediment concentrations in complex sediment matrices

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Background. Sediment porewater concentrations of hydrophobic organic contaminants measured by passive sampling are often used to indicate bioavailability and potential risks to aquatic organisms over more traditional bulk solid concentration measurements. Due to the cost and complexity of in-situ monitoring, the sorbents are often placed at a small number of locations at a given site and generally measure only a small volume of interstitial water. This leads to concerns that the individual measurements of passive samplers are less representative than bulk solids. The definition of uncorrelated areas, e.g. "hot spots", is also of interest for both solid and porewater concentrations. Objective. The aim of this study was to evaluate and compare the spatial correlations of in situ hydrophobic organic compound (HOC) porewater concentrations determined by passive sampling and the bulk sediment concentrations to evaluate representativeness and hot spots using geostatistical analysis at several contaminated sediment sites. Methods. Porewater and bulk solid samplers were analyzed from three different field studies conducted at Roxana Marsh, Grand Calumet River, Indiana, Hunters Point Naval Shipyard (HPNS), San Francisco, California and Columbia Slough, Portland, Oregon. Solid phase microextraction (SPME) fibers (approximately 30 µm polydimethyl siloxane (PDMS) coatings were preloaded with performance reference compounds (PRCs) inserted in shielded or unshielded holders, and embedded vertically into the sediment. After equilibrating the fibers were retrieved, sectioned and analyzed for PRCs, 15 PAH compounds or 111 PCB congeners using HPLC with fluorescent detector or GCTQMS. Surficial bulk sediment samples were taken from the same locations and the concentrations were also analyzed. The measured concentrations were subjected to statistical analysis including semivariogram analysis. Results. Data analysis showed that the spatial correlations typically exhibit an equal or longer range in porewater than in solid sediments, which suggest that point porewater measurements are potentially more representative of the surrounding area than bulk solid concentrations. The porewater is itself an integrative medium while HOCs in solids are often associated solely with the organic matter in the solids. The analysis was also used to identify spatially correlated versus uncorrelated (i.e. areas influenced by different sources) locations at the sites.

RP106 Measuring DDT, DDD, and DDE in sediments with passive sampling: implications for reactive compounds

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DDT and its degradation products, DDD and DDE, remain pollutants of concern at various sites in the world. Passive samplers are promising tools for evaluating freely dissolved concentrations of hydrophobic organic chemicals in sediment beds. In conjunction with performance reference compounds (PRCs), passive samplers have been successfully used to determine freely dissolved concentrations of PCBs and PAHs in contaminated sediment beds. However, using passive samplers for DDT, DDD and DDE poses the challenge that depending on the conditions in the sediment, DDT may be reacting to produce DDD and/or DDE. This reactivity can in turn affect the transport of PRCs and the ability to determine the freely dissolved concentrations of DDT, DDD and DDE using passive samplers. To address this issue, we developed a 1-D reaction-diffusion model for chemicals exchanging between PE and the sediment bed. While the transfer of non-reactive compounds such as PCBs was well described by existing 1-D diffusion models, the reaction-diffusion model better captured the transfer of the reactive DDT PRCs. We employed this reaction diffusion model for evaluating the fractional equilibrium of the native DDT, DDE and DDD in passive samplers deployed at a DDT contaminated site. We also used the reaction diffusion model to infer in situ degradation rates of DDT. The high in situ degradation rates determined from the DDT PRC loss suggested that the presence of parent DDT in the sediments is indicative of a recent or ongoing source of "fresh" DDT. Our results suggest that passive sampling in combination with mass transfer models of PRCs, could be used to obtain quantitative information about the biogeochemical processes governing the fate of contaminants. Freely dissolved concentrations in combination with degradation rates can then be used as inputs in larger scale mass balance models to inform decisions about risk management and remediation.

RP107 Cross-validation of equilibrium sampling and non-equilibrium passive sampling methods in PCB contaminated sediments

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Equilibrium and non-equilibrium sampling methods using well characterized polymers have been developed for measuring sediment porewater concentrations within environmental monitoring and site specific risk assessments. Straight-forward calculation of sediment porewater concentrations from polymer concentrations is possible when sediment slurries are allowed to equilibrate with polymer films in the laboratory (i.e., ex situ). In situations where in situ sampling is desired, non-equilibrium passive sampling methods may be preferable. In this case, mathematical models of Fickian diffusion are used to determine correction factors for the disequilibrium condition before conversion of polymer concentrations to porewater concentrations, which can introduce additional error and uncertainty. In this work ex situ equilibrium sampling with silicone coated jars and in situ non-equilibrium sampling methods low density polyethylene (PE) strips were used to determine porewater concentrations of polychlorinated biphenyls (PCBs) in New Bedford Harbor (Massachusetts, USA) sediments. Mesocosms were prepared by placing well-mixed sediment and water in glass aquaria. Strips of PE were inserted directly to the sediment bed while cores of the sediment were collected and subsamples of them tumbled in silicone coated jars. Freely dissolved concentrations measured by the two approaches were in very good agreement and on average differed by a factor of less than 2. Precision was higher for equilibrium sampling using silicone coated jars ($RSD_{avg} \leq 4.6\%$, n=9) compared to in situ non-equilibrium sampling using PE strips ($RSD_{avg} \leq 18\%$, n=3). These results support the use of in situ deployments when there is a need to capture concentration profiles in intact sediment beds while demonstrating the ease-of-use and precision of ex situ sampling methods.

RP108 Fatty acid binding protein in oyster Crassostrea gigas as a binding site to pharmaceuticals

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Toxic substances, including some polycyclic aromatic hydrocarbons (PAHs), pharmaceuticals, trace metals, among others, are frequently present in sanitary sewage and can be detected in estuaries, which may affect the homeostasis of organisms. Over the last two decades, pharmaceuticals, considered as emerging contaminants, have been receiving increased attention as potential hazardous to marine organisms. Some widely used pharmaceuticals, such as ibuprofen, paracetamol and diclofenac, can be assimilated by marine organisms and their effects in those species require more studies, particularly in sessile and filter-feeding organisms, such as bivalves. These sentinel organisms, such as Crassostrea gigas, have been used to evaluate environmental contaminant effects. Understanding the xenobiotic metabolism, such as pharmaceuticals substances, in this species is highly relevant for biomonitoring programs and oyster farming purposes. Previous studies have shown a significant up-regulation of the fabp gene which codifies one fatty acid binding protein (FABP) in C. gigas exposed to sanitary sewage effluents. FABP protein family possesses a hydrophobic pocked where fatty acids usually bind as primary ligands. The aim of this work was to evaluate the potential interactions of pharmaceutical with the binding pocket of a specific FABP isoform. We selected a C. gigas fabp isoform (ABU41520.1) homolog to human intestinal fabp, which has been previously proposed as a molecular biomarker of exposure to xenobiotics. Swissmodel and Swissdock softwares were used for modeling C. gigas FABP and molecular docking analysis of interactions to pharmaceutical substances (ibuprofen, paracetamol and diclofenac) and endogenous ligand (palmitate and oleate). All tested ligands showed negative free energy levels (ΔG) compatible with stable ligands. Particularly, palmitate and ibuprofen showed the most favorable interaction with the lowest energy. FABP protein was successful heterologously expressed in E. coli cells and purified by using affinity chromatography. Palmitate and ibuprofen, ligand-binding assays were carried out by fluorescence techniques (1,8ANS) and circular dichroism spectroscopy. Palmitate and ibuprofen appear to interact with the hydrophobic cavity of fabp. Our findings strongly suggest that fabp could be an ibuprofen binding protein in C. gigas after exposure to this contaminant in the environment. Financial support: CNPq.

Methods and Applications of Non-targeted Mass Spectrometry for Environmental Characterization

RP109 An integrated approach to study the formation and environmental behavior of transformation products from organic micropollutants

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A holistic assessment of environmental fate of organic micropollutants requires comprehensive information on their transformation products (TPs) since TPs may be less degradable and/or more toxic than the parent compounds. However, the fate of TPs has been scarcely investigated, much due to conventional target analytical approaches being limited by the absence of information on TP identities. The presented work addressed these knowledge gaps with a tiered workflow, with which we first identified TPs by combining laboratory incubations and non-targeted screening with high resolution mass spectrometry (HRMS), then investigated the behavior of identified TPs both in the lab and in situ. Water/sediment tests were carried out in bottles spiked with 9 pharmaceuticals. Water samples were taken and analyzed by UHPLC/QToF-MS under full scan data acquisition mode. A HRMS data-processing method was established, which enabled identification of 11 TPs that were formed with increasing concentration trends along the dissipation of their parent compounds. Experiments were then conducted using a flume to examine the behavior of TPs in a simulated river system. The same parent pharmaceuticals were spiked in surface water and the 11 TPs identified from the bottle incubations were monitored. Generally, TP formation and behavior were found consistent with the observations from the bottle incubations, indicating the TP information generated from static batch experiments can be qualitatively transferred to more complex systems. Thereafter, field studies were carried out in four rivers over a period of one week and the behavior of these 11 characteristic TPs was determined in a river stretch downstream of a sewage treatment plant (STP) outfall. The TPs formed in the lab-controlled experiments were also detected at the sampling sites close to the STP effluent, with the highest concentration of 0.89 µg/L found for metoprolol acid in a small river containing high proportion of treated

wastewater. Significant loss along the stretch was observed for most of the detected TPs. The results suggest STPs a pronounced source for TPs to surface water. TPs may be formed during wastewater treatment and then emitted into recipients, where they can undergo further transformation. Overall, the presented approaches provide a systematic workflow to identify environmentally relevant TPs and allow for comparisons between observations from lab-based experiments and field studies.

RP110 The use of non-targeted metabolomics profiling to study associations between POP exposure and biomarkers of human health in elderly California women

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Basic fundamentals of mass spectrometry have, in the last decade, allowed for a global characterization of the human metabolome, enabling researchers to study individual metabolic profiles at a new and unprecedented detail.More recently this approach has been combined with the analysis of environmental pollutants with the overall aim to study the associations between human exposure to persistent organic pollutants and biomarkers (i.e. circulating metabolites) of human health. Persistent organic pollutants (POPs) are known toxicants and have been linked to numerous adverse effects in humans. Metabolites in the human circulation (e.g. fatty acids, glycolipids) play a key role in human health and have been shown to be important in disease development. Non-targeted metabolomics provides a tool to unravel which processes are playing a key role in this development. Therefore, the main aim of this study is to investigate the associations between POPs concentrations and metabolic profiles in serum samples from a group of 325 elderly California women. POPs were determined using GC-HRMS and LC-MSMS and quantified using isotope dilution. Metabolites were determined using LC-QTOFMS and when possible quantified using isotope dilution. A broad range of metabolites were detected and further studies to perform metabolite characterization are ongoing. Preliminary results show that human POP exposure might be associated with lipid-related metabolites. This initial investigation demonstrates the use of non-targeted metabolomics profiling in providing a more complete understanding of the relationship between human POP exposure and biomarkers of human health. The views expressed herein are those of the authors and do not necessarily reflect those of the California Department of Toxic Substances Control.

RP111 Towards benchmark suspect screening and non-targeted analysis methods

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Suspect screening (SSA) and non-targeted analysis (NTA) methods offer new approaches to efficiently generate exposure data for a growing number of chemicals in commercial use worldwide, for which there is a dearth of information. High-resolution mass spectrometry (HRMS) provides a practical means to characterize xenobiotic chemicals in a variety of environmental and biological media efficiently and broadly. These relatively new approaches use a variety of analytical instrumentation, data processing methods, acceptance criteria, and reporting standards. We are conducting a round-robin collaborative trial to evaluate a range of approaches currently used in SSA and NTA. Three categories of experiments are being used: 1) ten standard chemical mixtures from the EPA's ToxCast library, 2) standardized environmental matrices, and 3) standardized environmental matrices spiked with known chemical mixtures. The ToxCast library mixtures include a structurally diverse set of compounds, focused on environmental chemicals with exposure potential. Extracts of standardized environmental matrices including house dust (NIST SRM 2585), human serum (NIST SRM 1957), and environmentally deployed silicone passive samplers are being provided to each participating laboratory to eliminate most aspects of pre-analysis variability. Gas and/or liquid chromatography with HRMS are used in an attempt to maximize chemical space coverage. Initial results will be discussed including (but not limited to): methods, software, and databases used; identified chemicals with level of certainty; percent correct identifications, false negatives, and false positives. Heat maps, concentration estimates and hierarchical clustering of the data will be used to prioritize chemicals to confirm in environmental samples, which will be enhanced by the ToxCast mixtures of known chemicals. The goal of this work is to produce benchmark methods for analytical, reporting, and data analysis to facilitate further analyses and identify areas for improvement.

RP112 Screening of Pesticide Residues and Fluorochemicals using LC-HR-MS/MS and Product Ion Libraries

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Pesticides and fluorochemicals pose a potential threat to environmental and human health. Modern LC-MS/MS analytical techniques allow quick and efficient screening for hundreds of pollutants with excellent sensitivity and selectivity to meet global reporting guidelines and regulations. Recent advancements in LC-MS/MS technology, including hybrid systems such as quadrupole-quadrupole time-of-flight (QTOF), now provide the ability to perform targeted and non-targeted screening in food samples on a routine basis. Here, we present results using a new method to identify pesticides and fluorochemicals in environmental samples using the SCIEX X500R QTOF system. For pesticide analysis, samples were extracted using a QuEChERS method (Quick, Easy, Cheap, Effective, Rugged and Safe), and extracts were diluted 10x to minimize possible matrix effects. For fluorochemical analysis, samples were diluted 1:1 with methanol and directly injected. LC separation was achieved using a polar modified reversed phase column and a mobile phase consisting of water/methanol and ammonium formate buffer. MS detection was performed using information dependent acquisition to simultaneously collect accurate mass MS and MS/MS information using positive and negative electrospray ionization. Target compounds were automatically identified in a variety of food and water samples by matching retention time (when available), accurate mass, and isotope pattern of the molecular ion and confirmed using MS/MS library searching with SCIEX OS software and SCIEX product ion libraries for pesticides and fluorochemicals.

Fate and Effects of Metals: Regulatory and Risk Assessment Perspective

RP113 Comparison of EPA Estimated vs. Field-Collected Inputs for the Biotic Ligand Model

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The Biotic Ligand Model (BLM) is a regulatory model widely used for estimating metal toxicity in aquatic systems, based on water chemistry and geochemical properties. Since these models rely on water parameters that are not always readily available, the USEPA recently released a draft Technical Support Document (TSD) which provided an approach for estimating missing water quality parameters to supplement incomplete data sets. The TSD presents calculated values for eight of the ten required parameters for BLM modeling, suggesting pH and temperature should always be measured, based on level III ecoregions and stream order. Although the TSD was immediately intended for criterion calculation and implementation for copper, this study compared BLM outputs for a suite of dissolved metals for which BLMs are currently available (Al, Cd, Cu, Ni, and Zn). Measured water quality parameters and metals concentrations of four first order streams located in the Blue Ridge ecoregion were used to perform BLM simulations, and the resulting criterion values were compared to EPA supplemented values. Outputs for dissolved metals showed differences within and among sites for both estimated and field-collected data.

RP114 Implementation options for water quality standards for nickel in Australia

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The European Community recently adopted a Water Quality Criteria (or Environmental Quality Standard, EQS) for nickel which is based on bioavailability, and uses a Biotic Ligand Model (BLM) based approach for its implementation. A programme of testing of the applicability of the BLM approach for nickel was undertaken in Australia in collaboration with local research organisations. Initial testing of nickel toxicity to Australian test species in field collected Australian surface waters revealed some limitation in the ability of the existing individual species BLMs to predict nickel toxicity in some of these waters. Testing was also performed on a soft water tolerant hydra which indicated increased importance of competition from hardness cations (Ca^{2+} and Mg^{2+}) in extremely soft waters (Ca < 2 mg l^{-1}). This study presents a comparison between several different possible approaches towards the implementation of a bioavailability based standard for nickel in Australia. Available possibilities for the derivation of an environmental threshold for nickel, and various other trace metals, include the use of a single value standard, a hardness corrected standard, a standard based on another single water quality parameter such as DOC, and a standard based on an integrated bioavailability approach similar to that applied in Europe. Each of these possibilities for the derivation of a standard for nickel are comsidered an dassessed against one another in order to identify which approach provides the most appropriate compromise between complexity and ecological relevance.

RP115 Mercury concentrations in fish from three major lakes in north Mississippi: Spatial and temporal differences and human health risk assessment

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The goal of this study was to compare total mercury (Hg) concentrations in fish muscle tissue and assess consumption risks of fish collected from three north Mississippi lakes (Sardis, Enid and Grenada) that are extensively used for fishing and recreation. Largemouth Bass (LMB; n=64, Channel Catfish (CC; n=72) and White Crappie (WC; n=100), that represent a range of trophic levels, were collected during Spring 2013 and 2014. Creel data estimated that anglers harvested approximately 370,000 kg of WC, 27,000 kg of CC, and 15,000 kg of LMB from the lakes annually. Median Hg wet weight concentrations were highest in LMB (443 ng/g), followed by CC (211 ng/g), and WC (192 ng/g). Fish-Hg concentrations were lower than those reported in fish >10 years ago. There were significant differences between lakes consistent across species. Grenada length normalized fish-Hg concentrations were higher than those from Enid and Sardis. Because existing consumption advisories for CC are length-based, the lack of relationship between length and Hg concentration indicated that the recommendations may not be sufficiently protective. Further, five different risk assessment paradigms yielded hazard quotient (HQ) values suggesting that existing fish consumption advisories may be insufficient to protect adults and especially children from exposure to Hg. Supported by U.S. Geological Survey and Mississippi Water Resources Research Institute.

RP116 Revising EPA's National Copper Ambient Water Quality Criteria for the Protection of Estuarine/Marine Aquatic Life Using the Biotic Ligand Model

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EPA's National Recommended Water Quality Criteria for aquatic life were first developed for copper in estuarine/marine environments in the mid-1980s and were last updated as draft criteria in 2003. Consistent with provisions in the Clean Water Act, National Recommended Water Quality Criteria are periodically revised to ensure they incorporate the latest scientific information and are protective of aquatic life. The 2016 update of the copper estuarine/marine criteria represents a substantial change in the way this criteria is calculated by incorporating use of the saltwater biotic ligand model (BLM), a bioavailability model that relies on water quality input parameters to estimate copper criteria that is protective of aquatic life in estuarine/marine environments. Incorporation of the BLM allows users to account for individual water quality variables (temperature, pH, dissolved organic carbon, and salinity) that influence the bioavailability and toxicity of copper in estuarine/marine environments. Use of the BLM will allow users to develop values that better target environmental protection by focusing on the fraction of copper that is bioavailable and can exert a toxic effect. In addition to the BLM, the 2016 update incorporates a more robust toxicity database supporting the updated criteria. A total of 78 genera are used to derive the 2016 estuarine/marine criterion maximum concentration in comparison to the 44 genera used for the 2003 draft criterion. Although limited additional toxicological data were available to directly derive an estuarine/marine chronic criterion, the acute-to-chronic ratio was revised using data for a broader range of marine and representative freshwater species to provide a more robust criterion continuous concentration value. In addition to cumulative nonpoint source releases, antifouling paints (used as coatings for ship hulls, buoys, and underwater surfaces) and CCA-impregnated timbers (used in decking, pilings and some marine structures) represent major sources of copper to the estuarine/marine environment, and the updated criteria will be beneficial for the protection of estuarine/marine aquatic life, particularly those occurring in and around coastal harbors and marinas. Details of the BLM approach, toxicological data used, and criteria derivation process are presented for this forthcoming criteria, which is scheduled to be published as final in late 2016.

RP117 Toxic effect of heavy metals from reused mine wastes on environment and human health

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With the current movement towards the increasing reuse of solid wastes such as mine waste in soil, it is necessary to understand leaching potentials of heavy metals from mine wastes that could contaminate environment, and the subsequent environmental and human health effects. The study investigates the leaching potentials of heavy metals from mine wastes and the ecological toxic effects and human health risk of heavy metals in mine waste leachates. Mine waste samples were collected from five metal mines (two different Pb-Zn mines, two different Fe mines, and a Mo mine) in South Korea. The Korea Standard Test Methods for Solid Waste (STM) and the synthetic precipitation leaching procedure (SPLP) were performed to figure out if heavy metal concentrations of leachates from mine wastes meet the regulatory levels for waste reuse in Korea. The SPLP was conducted to generate leachate that would be formed on exposure to acid rain. The heavy metal concentrations of the STM leachates and the SPLP leachates did not exceed the Korea regulatory levels. Based on a conservative assumption that these leachates flow into water streams, the leachate heavy metal concentrations were compared with the Korea effluent water quality standards, but they did not exceed the standards. However, one of the mine waste samples exceeded the standard for the toxic effects on Daphnia magna (TU>1). The Solubility/Bioavailability Consortium (SBRC) method was used to determine the bioaccessible forms of metals from mine waste. Carcinogenic and noncarcinogenic risks of the bioaccessible metals are to be calculated to see if the leachates impose any toxic effects on human. The leaching potential and ecological toxic effects show that a proper post-management of mine wastes such as heavy metal removal might be necessary to prevent harmful effects on environment. Overall, it is necessary to assess ecological and human health risk of reusing mine wastes in order to promote mine waste reuse.

RP118 Application of the Transformation/Dissolution Protocol to determine the rate of metal removal from the water column for chronic hazard classification

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The UN Globally Harmonized System (GHS) and the EU Classification, Labelling and Packaging (CLP) hazard classification schemes include the concept of degradation whereby rapid degradation from the water column (greater than 70% removal in 28 days) results in different classification cut-off values and categories. For metals and inorganic metal compounds, the rapid and irreversible removal from the water column is analogous to the rapid degradation concept for organics, recognizing that metal ions can be made non-available from the water column by a number of processes (e.g. precipitation and mineralization). This work is aimed at adapting the UN Transformation/Dissolution Protocol (T/DP) to establish the rate and extent of partitioning of metals from the water column so that the resulting data can be used in metals chronic classification. The T/DP is carried out under aqueous conditions representative of those generally found in the aquatic environment. Through the addition of sediments, data on water column residence times and processes can be generated. We have examined the rate of removal of Ni (1000 µg/L) and Co (500 µg/L) in the presence of two different sediments at pH 6. Following the addition of 1, 100 and 10,000 mg/L of either Raisin River (RRS) or Buffalo River (BRS) sediment to the individual T/D solutions, we agitated the jars at 100 rpm for one minute to suspend the sediments, then allowed the sediments to settle. At 24, 48, 96, 168, 336 and 672 h intervals, we then sampled the solutions for analysis of the dissolved concentrations of Ni and Co as a function of time. The results for the Raisin River sediment, which could be described as a medium grain sand with 0.8 C% and ~2% Fe, revealed 72% removal of Ni from the water column and 76% removal of Co for the 10,000 mg/L sediment loading over the 28 day period. The Buffalo River sediment, with 3.4 C% and 4.0% Fe, resulted in an 88% removal of Ni from the water column and 89% removal of Co for the 10,000 mg/L sediment loading over the 28 day period. Partition half-times ($t_{1/2}$) for Ni and Co for the 10,000 mg/L loading of RRS were 15 days for Ni and 13 days for Co. For the BRS sediment, $t_{1/2}$ was nine days for both Ni and Co. These values compare favourably with $t_{1/2}$ values calculated from experimental field data in freshwater lakes. The T/DP adapted to measure the rate of metal removal from the water column offers an experimental resolution to the issue of chronic classification for metals.

RP119 Evaluation of USEPA's draft "missing parameters" technical support document for application of the biotic ligand model

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The biotic ligand model (BLM) requires 11 input parameters for calculation of site-specific water quality criteria (WQC). At some sites, complete sets of BLM inputs may not be available, and this has raised questions about BLM applicability at those sites. In 2015, the United States Environmental Protection Agency (USEPA) released a draft technical support document that provides Level 3 Ecoregion default values for BLM inputs, with the intent of using these values when measured values are not available. The default parameters represent the 10th percentile values estimated on an Ecoregion-specific basis. We conducted an analysis to evaluate the effect of using some or all of the Ecoregion default values on BLM-based WQC for copper. Similarly, we evaluated the effect of the Ecoregion default parameters on BLM predictions representing the 5th percentile of chronic species sensitivity distributions (i.e., consistent with WQC) for lead and zinc. To evaluate the effect of using the default parameters on BLM predictions, complete sets of water chemistry data were obtained from the NWIS database for more than 1,000 sites around the United States. More than 20,000 complete sets of co-located (i.e. consistent in time and location) BLM inputs were compiled. Four scenarios of BLM calculations were evaluated: 1. WQC were calculated using reported values for all BLM inputs; 2. WQC were calculated by substituting Ecoregion-specific dissolved organic carbon (DOC) defaults; 3. WQC were calculated by substituting Ecoregion-specific calcium and magnesium; and 4. WQC were calculated by substituting all inputs (except pH and temperature). Results indicate that use of DOC defaults decreases the BLM-predicted WQC by generally less than 10-fold, but decreases of 100-fold were observed. Compared to DOC, use of calcium and magnesium default values had minor effects on BLM predictions. Lead and zinc predictions were more affected by calcium and magnesium substitution than were copper predictions. When default values were used for all parameters (except pH and temperature), BLM-based WQC were most conservative, although values were generally comparable to the scenario where DOC was the only substitution. These results suggest that it is advisable to use measured values for all BLM inputs, but that avoiding use of default values for DOC is most important.

Scientific Integrity Issues and the Applied Environmental Sciences

RP120 Removal of health care products in the tertiary polishing with constructed wetlands of conventionally treated municipal wastewaters

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Currently the use of personal and health care products has growth and intensified which in turn has also increased the release of the substances which fall in their composition to environment, especially to the water bodies. Since two decades ago these compounds have aroused the interest of scientific community because of the insufficient knowledge on their transport and fate in the acuatic environment and the insufficient information regarding their effects on the different trophic levels and in general in the health of the ecosystems. The objetive of this study was to evaluate an alternative for the tertiary polishing of the effluent from the conventional municipal water tretament systems, due to the fact that these systems do not remove some of these organic micropollutants with the required efficiency. With this purpose, at the end of the final sedimentation tank of the wastewaters treatment plant of the city of Izucar de Matamoros, were installed 15 constructed wetland (at mesocosm level) with different combinations of variables: Porous medium (Pm: gravel-G, medium and fine volcanic gravel TG and TF respectively), macrophyte (Pl: Phragmites australis,-Phr, Cyperus papirus- Cy, Thypa latifolia- Ty) and hydraulic residence time (HRT: 1, 3 and 5 days). To assess the removal capacity of the systems, the input and outputs loads of triclosan, diclofenac and naproxen were determined by gas chromatography coupled to mass spectrometry in 15 sampling campaigns. Removal percentages (PlPmHRT) were obtained between 38 (TyTF1) and 86% (CyTF5) for naproxen, 53 (PhrTG1) and 93% (CyTF5) for triclosan and 58 (PhrTG1) and 83% (CyTF5) for diclofenac. The statistical analysis showed a significant effect in the removal percentage of the three target analytes ($p < 0.05$) only for the HRT. However, removals above 57, 73 and 72% for naproxen, triclosan and diclofenac were obtained with different combinations of Pl and Pm for HRT 1. It is concluded that constructed wetlands are an efficient alternative for the tertiary treatment of conventionally treated municipal wastewater, improving its quality before its disposal to surface water bodies. Furthermore its construction materials can be tailored to the resources available in each region.

RP121 Designing, Performing, and Reviewing Toxicity Tests for Effective Presentation in Reports and Journals

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Arguably, the experimental design and performance of the toxicity tests are critical to the validity and usability of toxicity test results, but few consider the importance of peer-review in this process. The backbone of the scientific process is founded on the requirement of transparency of the methods and results employed such that another rearcher has sufficient information to reproduce the study. Often, individuals believe that the peer-review process is only needed to publish a paper in a peer-reviewed journal. It is often believed that research submitted to non-peer-reviewed journals and books, or research in stand-alone reports does not need the same degree of transparency and reproducibility. This is one reason that there is a mountain of poorly-executed studies with inadequate (if not incorrect) data and conflicting results that often leave data users baffled and confused. Screening the literature and only using studies from peer-reviewed outlets that are held to the highest levels is one way to address this concern; however, many chemicals or test species lack sufficient studies from peer reviewed sources. Although established practices, such as "Good Laboratory Practices" (GLP), typically result in using appropriate protocols and promote transparency through documentaion requirements, they may not always ensure that the study was adequately performed despite a solid track record of past performances. Through the use of case examples, this paper will discuss important considerations for the design and performance of toxicity tests as well as the presentation of the results, discussion, and conclusions. Additionally, we will recommend internal and external review strategies for all types of communication, not just papers for peer-reviewed journals.

PAHs in the Real World: Sources, Sinks, Bioavailability and Toxicity

RP122 A Comparative Developmental Toxicity Study on Embryonic Flounder Exposed to Crude Oils of Different Geological Origin

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Crude oils from different origins have distinct chemical compositions, hence might produce different toxicity. In the study, embryonic flounder were independently exposed to four crude oils, Basrah Light (Iraq), Pyrenees (Australia), Sakhalin Vityaz (Russia) and MFO-380 (processed fuel oil), with oiled gravel effluent. Total PAH concentrations were highest in MFO-380 (82,600 ng/mg), followed by Sakhalin Vityaz (25,600 ng/mg), Basrah Light (13,200 ng/mg), and Pyrenees (2100 ng/mg). Pyrenees was characterized by its relatively higher compositions of three to four ring PAHs, while two ring PAHs (C0 – C3 naphthalenes) highly dominated in Sakhalin Vityaz. MFO-380 and Basrah Light showed similar relative compositions except that Basrah Light had relatively high concentrations of dibenzothiophene and its alkylated homologues. Overall, the two types of crude oil (Basrah Light and MFO-380) produced highly similar gene expression pattern, primarily characterized by the well-known effects on biotransformation, immune response, cardiac function and oogenesis. Responses also included cardiac edema, spinal curvature and tail fin defect. High frequency percentage of cardiotoxicity and the low level of nkx expression were detected in embryonic flounder exposed to pyrenees which have higher composition of three to four rings. The expression level of CYP1A was highest in embryonic flounder exposed to Sakhalin Vityaz and MFO-380. This study has value in continued comparisons of crude oils from a wider range of sources. The results also indicate that differences in PAH composition from different crude oil origin can be an important factor understanding the impacts of other crude oil spills.

RP124 Ecotoxicological Risk of PAHs Released from Contaminated Sediments in Owen Sound, ON, Canada

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As a result of historical industrial activity, the sediments in the inner harbor of Owen Sound in Ontario, Canada are contaminated with PAHs, as well as other inorganic and organic contaminants. There is recent interest in evaluating the ecotoxicological risks associated with these in-place pollutants because of a proposal to dredge the harbor to restore navigation for deep-draught vessels in this Great Lakes port. We initiated a study to evaluate the levels and the distribution of PAHs and other contaminants in sediments in Owen Sound and the potential for these contaminants to be released into the water column at levels that are toxic to early life-stages of fish. Concentrations of total PAHs in sediments in the inner harbour were well above the sediment quality guidelines for the province of Ontario, with mean total PAH concentrations at the most contaminated site of 9,400 ppb dry weight. We deployed semi-permeable membrane devices (SPMDs) in the inner harbor to estimate the average concentrations of PAHs in the water column and the estimated concentrations for several compounds were above provincial water quality guidelines, including levels of fluoranthene, chrysene, benzo(a)anthracene and benzo(k)fluoranthene. In order to determine whether these contaminants in the water column are present at concentrations that pose a risk to fish, we exposed early life stages of two species of fish, Japanese medaka (Oryzias latipes) and lake whitefish (Coregonus clupeaformis) to extracts from the SPMDs deployed in the inner harbor. The medaka exposed to SPMD extracts developed yolk sac edema, which is a commonly observed developmental response in early life stages of fish exposed to PAH compounds. The whitefish did not show the same response, but these fry are currently being evaluated to determine whether they show more subtle toxicological effects.

RP125 Effects of dissolved organic matter on bioavailability and biodegradation of PAHs

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In environmental sciences, dissolved organic matter (DOM) is usually differentiated from particulate organic matter as the size fraction of organic matter smaller than 0.45 µm. This fraction typically consists of a multitude of structurally different compounds, all typically present at low concentrations, although it can differ significantly in quality (or biodegradability) and quantity in time and space. The environmental fate of organic pollutants, such as PAHs, can be affected by DOM via increased apparent solubility, desorption, transport and biodegradation. This overview presentation will focus on the recent research efforts in our group, where we have specifically addressed the influences of DOM quality on bioavailability-related phenomena, as connected with biodegradation of PAHs: chemotaxis, attachment and solubilisation. We used, for our studies, different experimental models to assess bioavailability, and these included Tenax extraction, dual 14C/residue analysis of microcosm samples, dynamic passive dosing with PDMS, biphasic NAPL/water systems, and column systems. Different model DOM sources, of dissimilar quality, were used, and included humic acids, root exudates, biosurfactants and organic fertilizers. We found that biodegradation of poorly bioavailable PAHs was enhanced by (bio)surfactants (Environ. Sci. Technol. 48:10869-10877, 2014), the targeted fertilization of free-oil phases or NAPLs (Environ. Sci. Technol. 45:1074-1081, 2011), by modulating the deposition and tactic motility of microbial degraders in porous media (Environ. Sci. Technol. 46:6790-6797, 2012), and by root exudates (Soil Biol. Biochem. 57:830-840, 2013; Environ. Sci. Technol.

49:4498–4505, 2015). However, a negative influence on biodegradation of PAHs by humic acids (Environ. Pollut. 184:435-442, 2014) and biosurfactants (Environ. Pollut. 205:378-384, 2015) was found if they prevented cell attachment to the PAH-loaded PDMS sources. These influences of DOM on bioavailability are relevant not only for innovation efforts in bioremediation but they have also connections with the determination of bioavailability of organic chemicals in retrospective and prospective risk assessment and regulation (Environ. Sci. Technol. 49:10255-10264, 2015).

RP126 Exposure to PAHs in Swedish Seafarers

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The purpose of this project was to investigate indoor air quality and measure personal exposure to polycyclic aromatic compounds (PAHs) on Swedish ships. Indoor air quality is important aspects of the environment which has not been studied extensively on ships. For seafarers' good indoor environment becomes particularly important as it represents both the working and living environment. On long trips that last for weeks to several months there is no way to change the indoor environment, and no exit from the ship. Air quality on a ship is characterized almost exclusively by the presence of substances that originate in the ship's fuel, lubricants and engine exhaust. Example of air pollutants are PAHs and this group of air toxics is of special concern since they may give an increased risk of cancer. Monitoring of PAHs is mainly studied with active sampling in workplaces. However, to simplify routine monitoring, passive air samplers may be the only option since they are cheap, easy to handle, and less disturbing. Best risk assessment is done using personal sampling. In this presentation, polyurethane foam (PUF) was used both as stationary, and for the first time as personal samplers for a three to seven days sampling period at different workplaces and on various job categories on a cruise ship. Measurements were carried out on two occasions: before and after the change of the fuel. We found a considerable wide range of PAH exposure levels for the various workplaces and occupational categories on the ship. However, all measurements were well below the Swedish occupational exposure limits (OEL) for benzo(a) pyrene and naphthalene. This study contributes to increased knowledge of exposure, sources and health effects to PAHs for Swedish seafarers.

RP127 Fate of polycyclic aromatic hydrocarbons in urban lake sediment

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The concentration of polycyclic aromatic hydrocarbons (PAHs) in urban lake sediments has increased in some catchments undergoing rapid urbanization by as much as two orders of magnitude over the last 20 to 40 years (Van Metre et al., 2000). One of the primary contributors to PAH loadings in urban lakes are coal tar pitch particles originating as runoff from paved surfaces, which were shown to significantly contribute to overall PAH loading in lake and streambed sediment (Van Metre et al., 2010; Yang et al, 2010). When these particles enter an urban lake the PAHs in the coal tar pitch are redistributed to more strongly sorbing carbonaceous materials (e.g., soot, charcoal), taken up by aquatic organisms, or released to the water column. An in situ tracer study was conducted in a lake near Milwaukee, WI to measure the fate and transport of PAHs in sediments originating from coal tar pitch. Sediment cores taken from the lake were amended with particles of coal tar pitch, asphalt, charcoal, and soot, and then placed back into the top layer of sediment. These cores were retrieved over a two year period and the exchange of PAHs between the different materials was quantified. PAHs were detected to move to and from all materials, with the greatest concentrations moving from coal tar pitch and asphalt particles, to the charcoal and soot particles. While soot and charcoal accumulated the greatest concentrations of PAHs, the sediment accumulated more mass than any other material. Transport of PAHs was measured to occur at least three times more in the horizontal direction (i.e. away from the cores) compared to the vertical direction (i.e. between different layers of the cores). Lab studies are being conducted to mimic field conditions under a more controlled environment.

RP128 Importance of Source Preparation and Selection Strategies in Chemical Mass Balance Modeling: Source Identification of PAHs in Urban Sediments

S. Ahn, Exponent, Inc. / Environmental and Earth Science; K. O'Reilly, Exponent / Environmental and Earth Science

EPA's Chemical Mass Balance (CMB) model is one of several receptor models used to identify the potential source(s) and quantify source contributions to receptor (environmental sample) concentrations. Originally developed by the USEPA for allocation of contaminants in air, CMB is now widely accepted and used for chemical apportionment in various media including sediment. The CMB model seeks a solution that can best fit the composition of chemicals in a sample via the linear sum of specified group of sources. Successful CMB application requires identification of the important sources that are relevant to the site under investigation along with the model's other underlying assumptions: source profiles are known and stable; and there are sufficient differences between sources so that they are linearly independent of one another. Ultimately, the value of the model's output depends on the quality of the source inputs. When CMB is applied for source identification and contribution of polycyclic aromatic hydrocarbon (PAH) contamination in urban lake sediment, caution is required in source selection strategy because there are many sources of PAHs in urban areas, and the PAH profiles of different sources are often similar. As a case study, CMB was used to identify sources and estimate source contributions to PAH-contaminated urban sediment impacted by multiple potential sources, including vehicle emissions, a coal-fired power plant, parking lot dusts, urban background sources, and more. This presentation demonstrates the impact of differently prepared and selected sources, and similarities between selected samples, on CMB model results. The relationship between the proposed sources and receptor samples is described using statistical tools such as principal component analysis and Pearson correlation. This case study provides an opportunity to review pros and cons of CMB application in PAH source identification in complex urban settings.

RP129 Incorporating contaminant matrix effects enables prediction of in vivo PAH bioavailability from in vitro digestor results

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Polycyclic aromatic hydrocarbons (PAHs) are carcinogenic pollutants found in soil worldwide. Soil physio-chemical properties have large variability, therefore predicting PAH bioavailability from ingested soil is an arduous task. Furthermore, individual PAHs have the potential to influence the physio-chemical properties, such as the solubility, of other PAHs. Here we quantify the solubility of 4 PAH compounds and compare single PAHs to multiple PAH mixture solubility in both water and bile fluids. Notably, the single compound solubility of benzo(a)pyrene and phenanthrene in either water or bile fluids were significantly ($p < 0.05$) different compared to multiple compound solubility. Given these results, the in vivo bioavailability matrix of 11 PAHs across 14 soils is compared to the in vitro bioaccessiblility matrix using co-inertia analysis to determine the principle PAHs affecting the bioavailability of other PAHs. Co-Inertia analysis reveals that the principle PAHs include benzo(a)anthracene, fluoranthene, chrysene, and anthracene. Including these PAHs as predictive variables in a linear model significantly improves the in vitro bioaccessibile predictive ability of in vivo bioavailability ($P < 0.05$). The predicted-observed linear models have a slope of 1.0, $p < 0.005$, and r^2 ranges between 0.65 for benzo(a)pyrene to 0.87 for anthracene. In conclusion, co-inertia analysis identifies the primary PAHs affecting PAH release from soil, allowing for improved bioaccessible predictions of bioavailability.

RP130 Lake-sediment record of PAH, mercury, and fly-ash particle deposition near coal-fired power plants in Central Alberta, Canada

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We report a historical record of atmospheric deposition in dated sediment cores from Hasse Lake, located near both current and previously operational coal-fired power plants in Central Alberta, Canada. The vertical distributions of spheroidal carbonaceous particles (SCPs), an unambiguous marker of high-temperature fossil-fuel combustion, polycyclic aromatic hydrocarbons (PAHs), and mercury (Hg) were used to evaluate the extent of contamination related to the coal-fired power plants in the region. Total organic carbon (TOC), C/N and stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope ratios of organic matter were determined to further examine anthropogenic inputs and changes in lake productivity over the past century. Accumulation rates of SCPs in the early part of the Hasse Lake record (pre-1955) compare well with the history of coal combustion to the east of the lake in the city of Edmonton, whereas accumulation rates in the latter part of the record (post-1955) suggest inputs from coal-fired power plants in the Wabamun Region to the west of the lake. Increasing accumulation rates of SCPs, PAHs, and Hg also coincide with the period of peak pollution in the Wabamun Region. Changes in TOC, C/N, and $\delta^{13}C$ and $\delta^{15}N$ ratios of organic matter indicate increases in lake productivity which may be related to anthropogenic atmospheric deposition.

RP131 PAH monitoring in the Great Lakes: What do dreissenid mussels tell us?

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NOAA's National Centers for Coastal Ocean Science Mussel Watch Program (MWP) has been using dreissenid mussels to monitor chemical contamination in the near shore zones of Great Lakes since 1992. Dreissenid mussels are abundant in the outer harbors on breakwaters and other hard substrates in the Great Lakes. These sessile, filter-feeders bioaccumulate contaminants, possess limited ability to metabolize contaminants, shed light on the bioavailability of contaminants to higher trophic levels, and serve as surrogates for benthic and wildlife health, thus making them an excellent tool for contamination monitoring and assessment. Beginning in 2009, MWP expanded its monitoring efforts by adding numerous sites within Areas of Concern and conducting place-based contamination assessments to address the issues under 'Toxics Substances and Areas of Concern' focus area under Great Lakes Restoration Initiative. MWP also began incorporating newer approaches such as the use of caged mussels and bivalve health metrics (biomarkers, genomics and metabolomics) into its routine protocol to aid contaminant characterization and link exposure to measurable effects in mussels. PAH is one class of compounds that have been routinely measured by MWP at all of its sites to date. We will present basinwide baseline PAH data from Great Lakes sites in relation to land use and population data, provide PAH distribution and fingerprinting results, and additional enhanced efforts of Great Lakes MWP achieved through numerous collaborations and partnerships with other federal, state, local agencies and academic institutions.

RP132 Polycyclic aromatic hydrocarbon accumulation in soil receiving rooftop runoff

S. Hileman, J.B. Belden, Oklahoma State Univ / Integrative Biology; J. Vogel, Oklahoma State Univ

Rooftop runoff, especially in urban areas, may carry contamination into the receiving soil. For instance, polycyclic aromatic hydrocarbon (PAH) contamination from atmospheric deposition or leaching from building materials may accumulate in the soils under downspouts or drip lines. The magnitude of contamination likely varies based on roof type and age of building. To gain a better understanding of the potential for PAH contamination, we measured PAHs in soil within 1m of drain spouts or roof edges at 92 structures, including single house residential buildings, industrial buildings, and institutions such as schools. More than 65% of the samples collected had higher levels of benzo[a]pyrene (a priority PAH) than the recommended soil screening level of 15.7ng/g. Several sites, which greatly exceeded soil screening levels, were locations where older buildings had roofing components covered with sealant, presumably containing coal tar. These preliminary findings are significant because buildings with particularly high PAH levels may put children at risk for additional exposure to carcinogenic PAHs, by either ingestion or contact with contaminated soil. These data also suggest potential contamination issues for harvested rainwater using rooftop runoff catchment systems.

RP133 Predicting Passive Sampler Equilibration Times for PAHs in an Amended Sand Cap

J. Roberts, SiREM; M. Vanderkooy, J.M. Conder, Geosyntec Consultants; M. Healy, SiREM; J. Thompson, C. Thompson, Geosyntec Consultants

PAHs in sediment pore water can be sequestered before upwelling water reaches the sediment-water interface using amended sand caps with amendments such as activated carbon and biochar. Passive samplers offer a method to monitor remedy effectiveness. A key condition for using passive samplers to directly estimate hydrophobic organic compounds (HOCs) pore water concentrations is that the concentrations of the HOCs being sampled are in equilibrium between the sampler and the pore water. The time to achieve equilibrium between the passive sampler and the surrounding pore water is dependent on both the hydrophobicity of the HOC and the surrounding chemical environment. The loss of performance reference compounds (PRCs) during deployment of a passive sampler allows for the estimation of the degree of equilibrium achieved or confirmation of the achievement of equilibrium. If the passive sampler is found not to be at equilibrium with the surrounding pore water, then the PRC data can provide an estimation of passive sampler uptake rates for calculation of the equilibrium pore water HOC concentrations. This presentation will show theoretical and empirical data demonstrating the difference in estimated equilibration times for various PAH compounds in different amended sediment cap environments. The equilibration times measured ranged from ~20 days in a powdered activated carbon (PAC) amended cap environment to ~200+ days in a sand only cap with no flowing water. Equilibration times were determined using deuterated PAH and hydrophobic dye PRCs. The results of the research can be used to determine if PRCs are required in passive sampling programs depending on the cap conditions (e.g. PAC or no PAC), HOCs of interested being measured and estimated sample deployment time.

RP134 The Application of Monoclonal Antibody-Based Biosensor Analysis for Quantifying PAHs in Sediment Pore Waters

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Hydrophobic contaminants such as polycyclic aromatic hydrocarbons (PAH) accumulate in sediments and may pose significant ecological and human health risk. Contaminant bioavailability is governed by partitioning but multiple chemical and physical factors influence bioavailability in natural systems. Because measuring contaminant exposure and uptake in biota is time consuming and expensive, multi-phase models have been developed to predict contaminant bioavailability. The heterogeneity of natural habitats makes it difficult to reliably predict bioavailability from measured bulk sediment concentrations and properties. Ultimately, site-specific pore water measurements are needed to accurately predict contaminant fate and bioavailability but these measurements are difficult to obtain by standard analytical methods. Advances in biosensor technology allows near real-time measurement of contaminants at sub ppb

concentrations in small volume (< 5 mL) aqueous samples. A quantitative, monoclonal antibody (mAb)-based sensor was used to measure PAH concentrations in sediment pore water collected at various PAH contaminated sites in the Elizabeth River, VA and Baltimore Harbor, MD. PAH concentrations in pore water samples were measured in the field within minutes of collection with the biosensor to map the spatial distribution of PAHs at contaminated sites in the Elizabeth River, VA. Sampling of pore water concentrations with depth by drive-point piezometer allowed vertical profiling of PAH concentrations within sediments to evaluate the input of contaminated groundwater and document mixing of surface water within the sediments. Analysis of PAH concentrations in pore waters from sediment cores collected in Baltimore Harbor was used to evaluate PAH toxicity to benthic amphipods. Correlations between PAH concentrations measured by the biosensor (< 1 µg/L to > 800 µg/L) and those measured by GC-MS were excellent and the results are being used to help develop sediment remediation strategies.

RP135 The Interactive Role of Fate Processes in the Overall Aquatic Fate of Polycyclic Aromatic Sulfur-Containing Hydrocarbons (PASHs)

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Crude oil spills release significant amounts of polycyclic aromatic sulfur-containing hydrocarbon (PASHs) and their alkyl homologous into aquatic systems. Dibenzothiophene (DBT) and its homologous, C1-, C2- and C4- DBT, were chosen as model compounds to investigate possible fate pathways of PASHs after a crude oil spill. Loss due to reaction with hydroxyl radical (indirect photolysis), volatilization from water and biological uptake were investigated by measuring their physicochemical properties. The EPA Exposure Analysis Modeling System (EXAMS) was used to estimate the relative impacts of these properties to overall chemical fate as a function of depth (10 cm, 1 m and 2 m) and salinity. The hydroxyl radical rate constant ($K_{\cdot OH}$), Henry's law constant (H), and n-octanol/water partition coefficient (K_{ow}) of PASHs were determined in distilled water. The K_{ow} which is an indicative of biological uptake, was also determined in artificial seawater to investigate the salinity impact on bioavailability of the PASHs. The analogue C1- DBT reacted faster with ·OH compared to other PASHs. The C2- and C4-DBT analogues had higher H compared to other homologues. Furthermore, PASH K_{ow}'s were increased as their molecular weight and water salinity increased. These results suggest that reaction with ·OH might be a significant dissipation pathway for smaller and more polar PASHs in shallow aquatic systems. In addition, high H and K_{ow} suggest that volatilization or uptake by aquatic organisms might be an additional potential fate pathway for high molecular weight and less polar PASHs. The overall half-lives of PASHs, assuming only dissipation due to indirect photolysis and volatilization, were estimated to increase as a function of depth in the water column. The decreasing order of half-lives of PASHs at 10 centimeters depth are DBT > C1-DBT = C4-DBT > C2-DBT which are 4.2 > 0.9 > 0.5 days, respectively. However, the decreasing order of half-lives of PASHs at 2 meters depth are changing to C4-DBT > DBT > C1-DBT > C2-DBT which are 15.1 >13.7 > 11.4 > 9.7 days, respectively. The C2-DBT half-life was estimated to be the shortest in either depths possibly due to higher contribution of volatilization than indirect photolysis in overall PASHs aquatic fate.

RP136 The polycyclic aromatic hydrocarbon 2-naphthol accelerates adipogenesis in the 3T3-L1 model

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Polycyclic aromatic hydrocarbons (PAHs) are known carcinogens and suspected endocrine disruptors. 2-naphthol is one of the important metabolites of naphthalene, which is the simplest PAH. Indeed, urinary 2-naphthol is used as a biomarker of exposure to PHAs in humans. Results from epidemiological studies show associations between exposure to 2-naphthol and obesity, a condition characterized by excess adipose tissue. In this study, we evaluated the effects of 2-naphthol on adipogenesis using mouse 3T3-L1 cells. These cells, under certain conditions, differentiate from fibroblasts to adipocytes, and are widely used for studying adipogenesis. Thus, 3T3-L1 cells were exposed to different doses of 2-naphthol. Cell differentiation was evaluated by cell morphology, lipid production, and mRNA expression of marker genes characteristic of either early adipocyte differentiation: CCAAT-enhancer-binding protein β (C/EBPβ), insulin receptor substrate 2 (IRS2), and sterol responsive element binding protein 1 c (SREBP1c); or terminal differentiation: C/EBPα, peroxisome proliferator-activated receptor-γ (PPARγ), and fatty acid binding protein 4 (aP2). Production of the antimicrobial peptide cathelicidin (Camp), which is produced by differentiating adipocytes and modulates inflammation and immunity, was also evaluated. Cell morphology changes and increased lipid accumulation indicated that 2-naphthol induced 3T3-L1 differentiation. During early differentiation, 2-naphthol stimulated C/EBPβ, IRS2, and SREBP1c expression, whereas during terminal differentiation, 2-naphthol increased C/EBPα, PPARγ, and aP2 expression. Moreover, 2-naphthol elevated Camp expression in a dose-dependent manner. In conclusion, our results show that 2-naphthol accelerates adipocyte differentiation and induce Camp production in 3T3-L1 cells, thus indicating that 2-naphthol might play potential roles in the development of inflammation and obesity.

RP137 Analysis of incense by headspace-gas chromatography-mass spectrometry (HS-GC/MS) and thermogravimetric-gas chromatography-mass spectrometry (TGA-GC/MS)

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Twleve incense samples, six stick incense and six dhoop style, from four different manufacturers were characterized by headspace-gas chromatography/mass spectrometry (HS-GC/MS) and thermogravimetric analysis-gas chromatography/mass spectrometry (TGA-GC/MS) under pyrolysis conditions. Burning incense is a popular practice around the world across all races, religions, ethnicities, nationalities, and ideologies. There are numerous studies which show the chemical composition of incense before burning or after burning. However, there are few studies that investigate the chemical composition both before burning and after burning. A chemical fingerprint for the incense before burning was determined using HS-GC/MS and many hazardous compounds were found. The TGA-GC/MS was used to determine the products of pyrolysis and combustion under helium and oxygen atmospheres respectively. The TGA-GC/MS analysis showed the presence of benzene for four out of six samples and the different chemical compositions of incense after thermo gravimetric analysis were suspected to be a result of distinct smoke-generating compounds used in the manufacturing of incense. This research provides preliminary evidence on the harmful emission products and their relationship to incense ingredients.

RP138 PAH Phototoxicity on Porites divaricata corals

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PAHs are a group of toxic substances, with a wide environmental distribution, produced by incomplete combustion processes of natuaral and anthropogenic origin. These compounds pose a risk to aquatic organisms because of its potential to produce phototoxicity when combined with solar UV radiation. Coral reefs are located in areas where the transparency of the water allows solar UV radiation to penetrate deep in the water column. In Mexico, the Caribbean Sea is a natural area of great value in terms of biodiversity, but the rapid tourism development threatens this ecosystem. In the present study the reef zone of Puerto Morelos, Quintana Roo, Mexico was chosen as a study area due to its environmental significance. The aim of this work was to evaluate the median lethal concentration (LC50) of anthracene, Benzo[α]pyrene and fluoranthene on Porites divaricata corals. Samples were collected at a depth of 1 m and transproted to the laboratory where 120 branches (1-2 cm long) were distributed in 10 aquarium. PAHs were dissolved in HPLC grade acetone to obtain the following nominal concentrations: 0.95 and 1.9 of benzo[α] pyrene, 15 mg/L, 30 mg/L and 60 mg/L of fluoranthene and anthracene plus two controls with acetone and two controls with seawater. Coral samples were kept in the aquariums for 10 hours inside the laboratory. Followed by a SUVR exposure period in which the aquariums were put in fiberglass tanks (0.7 x 2.0 x 0.6 m) with a constant water flow to maintain temperature at 30 ° C. In situ chlorophyll measurements were taken at 6:00 am and at noon using a pulse amplitude modulation fluorometer. After 6 days of experimentation signals bleaching or mortality were observed. Mortalities were used to determine LC50. Results showed that Porites divaricata presented a decrease in the photochemical efficiency, bleaching and mortality in the presence of environmentally relevant levels of SUVR, which means that in areas of high boating activities PAHs may represent a risk factor for corals living in shallow areas.

Engineering, Toxicology and Risk Assessment Guidance for Sediment Evaluation at Dam Removal Sites

RP139 Best practices for sediment assessment: Lessons from five dam removals in North Carolina

T. Augspurger, US Fish and Wildlife Service / Ecological Services

A decision to proceed with dam removal needs a case-by-case assessment of environmental and economic costs and benefits. One component is evaluating the chemical nature of accumulated sediments. In North Carolina, dam removal guidance notes the need to evaluate contaminants but there are not established methods. Accordingly, dam owners were reluctant to conduct expensive testing without a certain regulatory framework, and the lack of a framework hampered efforts to manage analytical costs. In five dam removal projects since 2004, we used the framework of the USEPA/USACE technical guidance manual on disposal of dredged material in inland waters to evaluate sediments. Assessment areas were defined as the stream-reach impounded by the dams, plus a one-mile buffer laterally and upstream consistent with the ASTM International Standard Practice for Environmental Site Assessments: Phase I Environmental Site Assessment Process. Evaluations start with a tier 1 assessment of existing information to assess the potential for current and historic contaminant inputs to the impounded reaches. GIS maps were populated with information from USEPA's Facility Registry System, Toxics Release Inventory and Envirofacts databases and records maintained by State water, air, and waste management agencies. Sites within the assessment area were screened-in or screened-out for further evaluation. For facilities that needed more inquiry, we gathered information on pollutants discharged and pathways to the assessment areas. Database reviews were augmented with review of any water and sediment chemistry data. We conducted a recon of each site and interviewed staff knowledgeable on the land uses and history. Results were synthesized for stakeholders with a recommendation on concluding assessment or proceeding through tiers of additional data collection, including sediment chemistry and toxicity testing to the extent necessary to inform decision-making. Absence of pollutant sources indicated little need for more work to characterize potential contaminants. Specific issues identified in tier 1 reviews guided any additional sampling. While there is no standard method for evaluating potential sediment contamination at dams, the step-by-step approach of synthesizing contaminant source data, sharing that with stakeholders for a discussion on how to proceed, and collecting site-specific sediment data if needed effectively and efficiently addressed concerns of regulated and regulatory entities.

Strategies for Assessing Chemicals for Endocrine Activity: Making the Best Use of Screening-Level Information

RP140 Behavioral toxicity of mixtures of endocrine disrupting chemicals in Zebrafish (Danio rerio) larvae

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The presence of endocrine disrupting chemicals (EDCs) in the aquatic environment can have severe effects on the health of aquatic organisms. Numerous anthropogenic EDCs, such as plasticizers, fire retardants and antibacterial agents, enter the aquatic ecosystems via effluents from wastewater treatment plants and land runoff. Many of these chemicals have been shown to have adverse effects on fish, including disruption of reproduction, as well as effects on neurodevelopment and brain formation. Previously, studies have mainly focused on single compound exposures or simple mixtures at high doses which does not reflect the complex mixtures of EDCs at low concentrations present in the aquatic environment. Hence, the challenge today is to assess the effects of more complex mixtures of EDCs in low doses, which is the basis for the EU financed Horizon 2020 project EDC-MixRisk. EDC- MixRisk is an interdisciplinary project where epidemiological and bio statistical methods have been used to identify EDCs that are correlated with endocrine disruption in two pregnancy cohort studies. EDCs linked to three different health domains (metabolism, reproduction and neurodevelopment) were identified and mixtures of these bad actors (phthalate metabolites, phenols and PFASs) were synthesized and are presently being tested in experimental model systems. The current study aimed to determine the behavioral toxicity of low doses of the EDC mixtures on zebrafish (Danio rerio) during early development. Alterations of larval behavior caused by the EDC exposure were studied as an endpoint for neurodevelopment since behavior integrates many biochemical processes and can be a sensitive endpoint for sub-lethal toxicity of endocrine disruptors. Zebrafish were exposed to sub lethal doses of the mixture targeting neurodevelopment (found to be correlated with adverse neurological effects in the epidemiological study) and larval locomotion was tracked using the ViewPoint ZebraBox revolution software. Additionally, quantitative PCR (qPCR) was used to determine the effects of the EDC mixture on the expression of thyroid related genes. Our results indicate that low doses of the neurological mixture caused adverse effects on the of behavior zebrafish larvae. Preliminary results also show that several of the thyroid related genes (including Deiodinase 2) are negatively impacted by the low dose exposure.

RP141 Considerations for Developing, Validating and Implementing Performance Based Test Guidelines

S.L. Levine, Monsanto Company / Global Regulatory Sciences; E.M. Mihaich, ER2

To manage teh ongoing development of test guidelines that evaluate the same endpoint, OECD developed guidance on a new concept known as Performance Based Test Guidelines (PBTG). The PBTG concept creates a grouping of test methods, with similar essential components and endpoints, to ensure that the methods have equivalent performance. This concept is not entirely foreign to ecotoxicologists, where many guidelines have historically included the option of testing any one of a number of species provided they adhered to specific performance standards. However, it is a relatively new concept for in vitro assays and it is being implemented to manage the proliferation of in vitro test systems. In addition, EPA is interested in adopting performance based standards to manage acceptance criteria for high-through-put in vitro screening assays that will inform the EDSP. An overview and analysis will be drawn from the two initial PBTGs developed by OECD, the estrogen receptor transcriptional activation assays and the in vitro skin corrosion assay. This presentation will provide an overview of the general approach to implement PBTGs and standards, examples of assay types where PBTGs have been applied, approaches and considerations to cross-validate mechanistically and functionally equivalent "me too" assays, learnings from these first two efforts, and a discussion of advantages and disadvantages to applying PBTGs.

Chemical and Microbial Environmental Health Threats Associated with Disasters

RP142 Comparison of Wastewater-Associated Contaminants in the Bed Sediment of Hempstead Bay, New York, before and after Hurricane Sandy

S.C. Fisher, P.J. Phillips, USGS / New York Water Science Center; B.J. Brownawell, Stony Brook Univ / School of Marine and Atmospheric Sciences; J.P. Browne, Town of Hempstead / Dept of Conservation and Waterways

Wastewater infrastructure situated along the coast proved particularly vulnerable to coastal storms. Inundation brought by Hurricane Sandy caused widespread failures and overloads of wastewater treatment systems resulting in the discharge of billions of gallons of untreated and partially-treated sewage into the sensitive ecosystem of Hempstead Bay, New York, in the weeks following landfall. To assess the impact these discharges may have had on the benthic environment, bed sediment samples from sixteen sites collected and archived before the storm (2010-12) were analyzed and compared to samples collected after the storm (2013). Samples were analyzed for 74 compounds that are typically associated with wastewater, including sewage tracers (skatole, acetophenone, and indole), polycyclic aromatic hydrocarbons (anthracene, fluoranthene, and phenanthrene), human [steroid] hormones (3-beta-coprostanol and estriol), and personal care and domestic use products (caffeine and bisphenol a). Data from eight sites were used for statistical comparisons (Wilcoxon signed-rank test) of pre- and post-storm conditions—data from an additional eight sites with only pre- or post-storm samples were evaluated for regional context. Results from the most frequently detected wastewater tracers and ratios of steroid hormone and of polycyclic aromatic hydrocarbon concentrations indicate an increased sewage signal near the Bay Park sewage treatment plant outfall in Reynolds Channel (median percent change of +116% using 23 paired compounds) and downstream of Powell Creek, where raw sewage was discharged during emergency clean-up (median percent change of +240% using 24 paired compounds). At four sites further from the outfall, median concentrations were lower in samples collected after the storm (median percent change ranging from -70% to -25%), possibly because of sediment redistribution observed throughout the bay. The final two sites in the comparison did not have statistically significant differences in concentrations of compounds compared; however, relative to other sites, concentrations of wastewater tracer compounds were higher in both pre- and post-storm samples. Overall, this analysis indicates changes in bed sediment quality of Hempstead Bay resulted from a combination of additional sewage inputs, sediment redistribution, and stormwater runoff carrying sewage overflow and burned and unburned petroleum products in the days and weeks following the storm.

RP143 The impact of onsite wastewater disposal systems on groundwater in areas inundated by Hurricane Sandy in New York and New Jersey

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Thousands of coastal onsite wastewater disposal systems (OWDS) were inundated by Hurricane Sandy's storm tide. Inundation of community infrastructure or OWDS facilities during coastal flood events can damage OWDS, and may increase the load of poorly treated sewage into the shallow groundwater flow system and adjacent coastal waters. The presence of OWDS in areas inundated during Hurricane Sandy in 2012 has raised concerns over whether saltwater inundation affected 1) the presence of wastewater-associated compounds in the near shore shallow groundwater flow system and 2) the ability of the shallow groundwater flow system to attenuate wastewater-associated compounds. The high hydraulic conductivity of the sandy surficial aquifer of the New Jersey-New York coastal region makes these areas particularly vulnerable to organic wastewater contamination. This study compares the shallow groundwater quality downgradient of OWDS before and after Hurricane Sandy in the barrier island communities of Fire Island National Seashore, NY, and establishes a baseline for wastewater influence on groundwater in other NY (Long Island) and NJ (Sandy Hook) coastal communities inundated by Hurricane Sandy. Nutrients and contaminants of emerging concern (CECs; such as pharmaceuticals and hormones) were detected in shallow groundwater downgradient of OWDS in two settings along the New Jersey and New York coastlines: 1) a single, centralized OWDS serving a park facility; and 2) multiple, single-family home OWDS (cesspools) in areas with land use ranging from low-density residential to mixed use (residential and institutional). The most frequently detected pharmaceuticals were lidocaine (40%), carbamazepine (36%), and fexofenadine, bupropion, desvenlafaxine, meprobamate, and tramadol (24-32%). Land use settings with the greatest influence on downgradient wells were in locations that catered to the greatest number of people, regardless of the type of OWDS (centralized or cesspools/septic systems). These data demonstrate the importance of analyzing for a wide variety of CECs in regional studies to account for varied demographics. Comparisons between groundwater data collected before and after Hurricane Sandy at Fire Island, NY, show increases in the number of pharmaceuticals and the total concentration of pharmaceuticals after Hurricane Sandy, but those increases may reflect other factors (seasonality, usage) besides inundation.

RP144 Regional assessment of persistent organic pollutants in resident mussels following Hurricane Sandy

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Resident mussels are effective indicators of ecosystem health because contaminant concentrations in their tissues quickly respond to changes in ambient environmental levels, accumulation occurs with little metabolic transformation, and more than 20 years (1985-2012) of historical contaminant data within the United States are available. Mussels have been used worldwide to monitor changes in contaminants in the coastal environment but results from this and other studies suggest that they also have the potential to detect short and long-term impacts of episodic events as well as long-term impacts of incremental changes (sea level rise and land-use change). Blue and ribbed mussels were collected from 10 previously studied locations approximately a year after Hurricane Sandy and analyzed for a suite of persistent organic pollutants (POPs) including polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), polycyclic aromatic hydrocarbons (PAHs) and organochlorine pesticides (OCPs). A subset of shells from each site was also aged by thin-sectioning to determine if there were site-specific or local differences in mussel populations. No relation between length or age and latitude was observed indicating a similar distribution of ages across the sampled mussel populations. Regionally, concentrations of PCBs and PAHs decreased significantly, while concentrations of OCPs remained unchanged, and PBDEs significantly increased compared to historic concentrations. Although concentrations of PCBs, OCPs and PAHs were at or near record low concentrations, regional trends did not significantly change after Hurricane Sandy. At some locations, however, observed concentrations were an order of magnitude lower than pre-Sandy concentrations, indicating that the current study did provided a new baseline in POPs for select areas following a major coastal storm. A combination of tissue analysis, chronology and histology immediately following a major event (e.g. natural disaster) would be valuable and necessary to establish a link between contaminants and the resulting ecological effects. This study demonstrates that to effectively measure storm induced impacts it is necessary to understand the factors influencing changes in mussel body burdens, have a long-term monitoring network, and to have the ability to mobilize quickly post event.

RP145 Resetting the Bar: Establishing Baselines for Persistent Contaminants after Hurricane Sandy in the Coastal Environment of New Jersey and New York, USA

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Hurricane Sandy (often informally referred to as "Superstorm Sandy") was the second costliest hurricane to make landfall in the United States since 1900. In the immediate aftermath of Hurricane Sandy, it was readily apparent that long-term contaminant threats were possible due to a myriad of factors including the extent of compromised infrastructure, beach erosion, and sediment disturbance and the proximity of the storm track to population centers and vulnerable ecosystems. Although supplemental funding sources became available to U.S. Geological Survey (USGS) many months after the storm, scientists applied retroactive approaches to assess the potential contaminant impacts to public spaces and ecosystems. These approaches included: 1) the regional collection of environmental samples in locations (estuaries, bays, beaches) and media (sediment and tissue) including stations sampled prior to Hurricane Sandy; 2) the assessment of specific sources of contaminants particularly partially and untreated wastewater, 3) the potential for mitigation activities themselves to exacerbate the release of contaminants in, and near, public spaces; 4) the novel application of young-of-the-year bluefish as a sentinel species of contaminant mobilization and uptake; and 5) the evaluation of new remote sensing platforms to identify contaminant plumes in the environment. The results of these studies will published in a forthcoming special issue of Marine Pollution Bulletin providing resource managers and researchers with a comprehensive baseline dataset describing post-Hurricane Sandy contaminant levels throughout coastal New Jersey and New York and insights into the impacts of this event on wildlife and the built environment. Progress on the USGS strategy to define baseline and post-event sediment-bound environmental health stressors (SCoRR) and the ongoing assessment to compare storm-derived changes to long-term trends in sediment quality within the region in collaboration with the U.S. Environmental Protection Agency will be presented.

Integrating Chemistry and Biology in a Landscape Context to Reveal Potential Causes of Endocrine Disruption

RP146 Strategies to Address Endocrine Disruption in Fish and Wildlife in the Chesapeake Bay Watershed

K.L. Smalling, USGS / NJ Water Science Center

U.S. Geological Survey (USGS) scientists have established a national framework to evaluate endocrine disrupting chemicals (EDCs) and their effects on fish and wildlife. The four strategic goals for EDCs outlined by the framework were to 1) identify and quantify sources, fate, transport, distribution and exposure; 2) evaluate their effects on fish and wildlife; 3) determine their mechanism(s) and thresholds for adverse effects and 4) develop appropriate assessment tools and models to evaluate risk. The framework and strategic goals were applied directly to ongoing research in the Chesapeake Bay watershed to assess the exposure and potential effects of EDCs on fish and wildlife. The Chesapeake Bay is the largest estuary in the United States, and provides critical resources to fish, wildlife and people that use the 64,000 square mile watershed. For more than a decade, adverse effects associated with exposure to EDCs have been observed including intersex (testicular oocytes) in bass and plasma vitellogenin in male fishes (bass and sucker species). Skin lesions and mortalities of both adult and young-of-year bass have also been observed in fish from the same locations where the prevalence of intersex was high. Currently, emphasis is being placed on aquatic ecosystems with a focus on the identification of relevant EDCs, how they enter waterbodies, and how they affect aquatic organisms. Studies are investigating key pathways of EDC transport and exposure including the mechanisms and chemical thresholds associated with observed effects. Controlled laboratory and environmental field sampling approaches are being applied in tandem at six integrator sites that are dominated by agricultural land use. EDC research in the Chesapeake Bay Watershed was designed as a coordinated and collaborative effort between Federal, State and academic research partners to fill data gaps and synthesize findings. This study will provide a scientific basis for resource managers to consider strategies to reduce the occurrence of EDCs and their effects on fish and wildlife in this, and other, valuable ecosystems.

RP147 Effects of early life stage exposure of largemouth bass to a common Chesapeake Bay contaminant (atrazine) or a model estrogen (17α-ethinylestradiol)

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Field studies have shown high prevalence of intersex in smallmouth bass (Micropterus dolomieu), and to a lesser extent largemouth bass (Micropterus salmoides), populations in the Chesapeake Bay watershed. This observed phenotype has been correlated with the presence of the agricultural pesticide atrazine, which is widely used and one of the most commonly detected pesticides in the watershed. Our objective was to investigate the mechanisms of intersex formation in largemouth bass (LMB). An experiment was conducted in which LMB were exposed to either atrazine (1, 10, or 100 μg/L) or the model estrogen 17α-ethinylestradiol (EE2, a strong estrogen known to induce intersex and sex reversal in fish; 1 or 10 ng/L). Fry were exposed directly after hatch from 5 days post spawn (dps) through early gonad development (80 dps). The initial histological analysis of sex ratios indicated the 10 ng/L EE2 treatment resulted in all females indicating complete male sex reversal; however, there was no apparent sex reversal at 1 ng/L EE2. Additional analysis of sex identification by the presence of SOX9 and β-CATENIN as markers of "male" and "female" gonadal tissue, respectively, will be compared to histological results to determine any potential effects on sex differentiation. Future analysis will focus on potential biomarkers of intersex development and endocrine disruption (e.g., vtg, cyp19a, vasa, dazl, zp3, sf1, cyp17,dmrt1, foxl2). This study will shed light on the sensitivity of LMB to estrogens during sex differentiation, and potential effects of atrazine on gonad development in this species.

RP148 Time course of transcriptomic alterations in the adult fathead minnow (Pimephales promelas) ovary in response to atrazine exposure

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Agricultural land use is associated with increased concentrations of nutrients and pesticides in surface waters, as well as symptoms of endocrine disruption including reproductive abnormalities and disease outbreaks in resident populations. The herbicide atrazine is the second highest-use pesticide in the United States and is frequently detected in surface waters of the Chesapeake Bay watershed. Atrazine has been implicated in reproductive dysfunction and decreased egg production in fathead minnows (Pimephales promelas) during 30 d exposures at concentrations of 0.5, 5, and 50 μg/L. Here we evaluated the time course of transcriptomic alterations in ovarian tissue of adult fathead minnow exposed to 50 μg atrazine/L for 4, 14, or 30 d. Our objectives were to evaluate possible adverse outcome pathways underlying the observed reduction in egg production and identify putative biomarkers of exposure for future evaluation in the field. The ultimate aim is to determine whether the concentrations of atrazine observed in the Chesapeake Bay watershed could be contributing to declines in health of fish and wildlife. We observed over-representations of genes with altered expression in gene ontology categories including lipid metabolism, steroid metabolism, cell surface receptor signaling pathway, cell adhesion, and signal transduction. These changes suggest a challenge to regulatory feedback loops of the hypothalamus-pituitary-gonad axis. Future investigations will focus on candidate adverse outcome pathways involving the maturation-inducing hormone pathway, cell membrane estrogen receptor pathway, and SF-1 pathway.

RP149 Assessing the Effect of Early Life Exposure to EE2 on the Innate Immune Response of Largemouth Bass

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Exposure to estrogenic endocrine disrupting compounds (EDCs) such as 17a-ethinylestradiol (EE2) has been associated with reproductive failure and developmental abnormalities in both largemouth and smallmouth bass in US watersheds. In this study, we addressed the impact of EE2 exposure on the initiation of innate immunity in largemouth bass larvae using RNAseq. For the first 70 d of life, largemouth bass larvae were either exposed to an environmentally relevant concentration of EE2 (1ng/L) or vehicle control. On day 70 pf, fish were then injected with either bacterial or viral mimics or saline for 24 hrs while still in the presence of EE2. Individual hepatic mRNA libraries (n=18, PE200) were sequenced at a depth of >40M reads which were then used for de novo assembly to generate a reference transcriptome for largemouth bass. The hepatic transcriptome consisted of over 130,000 contigs, which upon Blast2GO annotation against other fish led to the prediction of roughly 35,000 ORFs. The reference transcriptome was then used to measure differential expression of the control and experimental groups. Initial analysis of EE2 exposure alone to vehicle control demonstrated that over 630 mRNAs were differentially expressed ($p < 0.05$). Current research includes assessing and validating genes and pathways involved in innate immunity that were dysregulated upon stimulation with the microbial mimics and how chronic EE2 exposure modified the expression of these genes. The availability of the annotated reference transcriptome for largemouth bass will complement studies designed to assess how EDCs may lead to increased disease susceptibility.

RP150 Determining the estrogenicity of wastewater effluents and low concentration hormone treatments in chinook salmon and their recovery following exposure

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Considerable information exists about the estrogenicity of waste water effluents (WWEs) and their potential to feminize male fish; however, WWEs in many jurisdictions have not been characterized with respect to their constituents, estrogenicity, or potential for environmental impacts to aquatic life. In this study, two tertiary waste water treatment plant (WWTP) effluents from British Columbia, Canada were evaluated. Little is known regarding the estrogenic potential of these complex mixtures; initial chemical analyses indicated estrone (E1) and 17α-ethinylestradiol (EE2) may be present. Both WWTPs discharge to freshwater environments where culturally and economically-important Pacific salmon spawn, hatch, and spend the majority of their freshwater life stage as juveniles before migrating to the ocean. This study had two goals: (1) to determine the contribution of natural or synthetic estrogen hormones to the overall estrogenicity of the WWEs, and (2) to evaluate the effects and recovery of juvenile chinook salmon exposed to either these WWEs or pure estrogen hormones. Chinook were exposed to environmentally-relevant concentrations (1% and 5%) of WWEs for 7 d and then transferred to clean water to recover for a further 7 d. The WWEs were evaluated for sterols (hormone) composition and chinook were then exposed (same exposure regimen) to similar estrogen hormone concentrations: E1 (2 ng/L), EE2 (0.5 ng/L, the local water quality guideline), 17β-estradiol (E2; 1 µg/L, +ve control). Using hepatic vitellogenesis-related transcript expression, the estrogenicity of the E1 treatment was shown to be similar to one of the WWEs in terms of estrogenic potential. Numerous molecular and biochemical endpoints were evaluated to examine the ability of fish to recover from hormone or WWE-induced effects. Fish were able to recover from alterations to liver somatic index, and plasma [glucose] and [lactate]. However, endpoints that remained/became altered after 7 d of recovery in clean water included gill Na^+/K^+ ATPase activity, [leukocytes], gonadal somatic index, plasma [vitellogenin protein], and hepatic transcripts of estrogen receptor isoforms, vitellogenin and vitelline envelope proteins. These results show that not all altered endpoints in fish exposed to WWEs and estrogen hormones recover in the short term following exposure, which indicates the health and fitness of juvenile salmonids could possibly be impaired by environmentally-relevant WWEs or hormone exposures.

RP151 Are responses of wild fish to wastewater effluent consistent? An assessment of spatial and temporal variability of biological endpoints

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It is well established that exposure of aquatic organisms to endocrine disrupting chemicals (EDCs) contained in municipal wastewater effluent (MWWE) can have deleterious effects on the health of fish populations. While this has been well demonstrated in the laboratory, it remains difficult to associate EDCs in MWWE with impacts on the health of wild fish populations. Our ability to detect change in exposed ecosystems is currently limited by our poor understanding of how responses to EDCs vary across levels of biological organization. The purpose of this study was to evaluate biological indicators of EDC exposure by observing their annual variability in a wild population of fish exposed to MWWE. The system used to conduct this study was the central reach of the Grand River in Kitchener, Ontario, where two municipal wastewater treatment plants are located. Rainbow darter (Etheostoma caeruleum) were collected from sites across an urban gradient in the spring and fall between 2007 and 2012. Analysis of selected pharmaceuticals, liver gene expression, gonadal sex steroid production, gonad histology, and somatic indices were compared across years and seasons. We found that in this system the consistency of biological responses varies according to the level of biological organization. Exposure and biological measures at the lower levels of organization (e.g. gene expression) are highly variable while measures that were at the middle of the biological scale (e.g. gonad histology) were the most consistent between seasons and years. Although endpoints representing higher levels of biological organization (e.g. somatic indices) are desirable because of their relevance, they are influenced by natural variability and more difficult to link to specific stressors. The findings of this study have important implications for future research efforts on environmental impacts of contaminants, as well as for the design and interpretation of biological monitoring programs globally.

RP152 Effects of thyroid hormones, thyroid hormone disruptors, and treated wastewater effluent on chemosensation in Lithobates catesbeianus tadpoles

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Endocrine disrupting compounds (EDCs) represent a large group of chemicals that have widespread use in our everyday lives, ranging from pharmaceuticals to personal care products. Consequently, EDCs enter municipal wastewater but are not fully removed by conventional wastewater treatment processes. Therefore low concentrations of EDCs are persistent in treated wastewater effluent. These low concentrations of EDCs in effluent may be biologically active and disruptive to the endocrine system in aquatic vertebrates. Some EDCs are able to disrupt the normal functioning of the thyroid system in vertebrates through agonism and antagonism of thyroid hormones (THs). Tadpoles are valuable sentinels for studying the effects of TH disruption because THs are the main drivers of metamorphosis. During metamorphosis the whole tadpole body plan is remodeled as the developing tadpole prepares for a semi-terrestrial and semi-aquatic lifestyle as an adult. In particular, THs trigger major structural and functional changes in the whole olfactory system. Therefore, TH disruption could lead to changes in chemosensory acuity in tadpoles. In this study, we exposed premetamorphic North American bullfrog (Lithobates catesbeianus) tadpoles to environmentally relevant concentrations of one of THs thyroxine (T_4), triiodothyronine (T_3); a cocktail of known EDCs; treated municipal wastewater effluent; or a negative control of 17β-estradiol (E_2). Chemosensory mediated avoidance responses to a stimulus (mixture of amino acids) were subsequently measured using an I-maze choice assay. The results show that T_3 impaired avoidance responses to the stimulus, but conversely T_4, E_2 and the cocktail of known EDCs had no effect on behavioral responses. Exposure to treated wastewater effluent however, did impair the chemosensory mediated avoidance responses to the stimulus. These results indicate that EDCs present at low concentrations in treated effluent may be able to mimic T_3, as avoidance responses to the stimulus were disrupted in both these treatments. It is important that chemosensory function remains intact in tadpoles, as it allows them to locate food and avoid predators. Therefore, chemosensory mediated behavior can serve as a sensitive environmentally relevant endpoint to detect TH disruption, and can be informative of the potential effects of EDCs in treated wastewater effluent receiving waters.

RP153 Toxicity of Triphenyltin on the Development of Retinal Axons in Zebrafish at Environmentally Relevant Concentrations

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The impacts of triphenyltin (TPT) on ecological health are of particular concern due to the unexpectedly high levels found in wild fish around the world. Here, zebrafish embryos were exposed to TPT via in ovo nano-injection to study its toxicity on the development of retinal axons in fish. Lipophilic dye labeling revealed obvious defects in retinal axon development in larvae with normally shaped eyes, with incidences of 0, 1.08%, 2.66%, 4.26%, and 6.85% observed in the control, 0.8, 4.0, 20.0, and 100 ng/g ww exposure groups, respectively, showing a dose-dependent increase. Larvae with retinal axon development defects exhibited dark coloration, indicating vision loss. Since the lowest observable effective concentration of TPT to induce retinal axon development defects was 0.8 ng/g ww, which is lower than the concentrations in wild fish eggs, this defect could occur in wild fish larvae. Alterations in the expressions of pax6 and ephrinBs, which regulate the establishment of retinal polarity, were correlated with defect incidence. Expression levels of the CYP26A1 gene and protein were significantly up-regulated in all exposure groups, which may lead to significant decreases in concentrations of retinoic acid (RA) precursors, retinol and retinal, though the sensitivity of the present LC-MS-MS method was insufficient to detect the concentrations of RAs in the zebrafish larvae. Such a disruption of retinoid homeostasis would, at least partly, contribute to the incidence of developmental defects in retinal axons. This study is the first to report that TPT at environmentally relevant concentrations can interfere with development of retinal axons in fish.

"One Health": Opportunities for SETAC Leadership in Integrating Environmental, Human and Animal Health

RP154 A One Health Approach To Study Mercury In Traditional Foods Of Bigstone Cree First Nation (Alberta, Canada)

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Mercury (Hg) is a global pollutant of concern since it can affect public health and ecosystem quality. First Nations communities are particularly susceptible to mercury contamination given that their traditional foods, which play a significant role in their health, culture, recreation, and spirituality, are known to be contaminated with mercury. Most studies concerning mercury contamination in First Nations communities have focused on seafood, with relatively few studies on other food sources. Further, there can be great variability in mercury contamination across communities, and thus there is a need for site-specific information. The objective of this study is to characterize mercury (total and methylmercury) levels in 150 traditional food items of concern to the Bigstone Cree Nation (Alberta, Canada). Through a participatory research activity, we identified and collected 13 different categories of animal and plant items of concern (Labrador tea, Berries, Rat root, Moose, Soil, Mountain-ash, Grouse, Hare, Fish, Mint, Duck, Water lily and, miscellaneous meat and plant samples). The food samples were processed, dried, and then analyzed for total mercury using a Direct Mercury Analyzer (DMA-80). In the plant samples the highest average mercury levels were in old man's beard (145.7 ppb) followed by Labrador tea (20.7 ppb) and mint (20.5ppb). Analyses of the meat samples are currently in process, as well as the methylmercury determinations. The work is particularly suited for analysis through a One Health perspective, a perspective shared by Aboriginal communities worldwide. As such, the data will be analyzed with respect to potential impacts on humans, organisms, and ecosystems.

RP155 Quantitative analysis of 296 antibiotic resistance and related genes in human impacted environments

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The origins of antibiotic resistance in the environment is relevant to human health because of the increasing importance of zoonotic diseases as well as the need for predicting emerging resistant pathogens. Antibiotics are used in diverse settings for food production. Domestic animals are treated with antibiotics for both curing disease and promoting growth. Moreover, aquaculture relies on antibiotics to manage infectious disease. Wastewater treatment plants receive sewage from various sources, including hospitals and households which are both important sources of antibiotics and their residues, and antibiotic resistant bacteria. We have characterized the antibiotic resistance gene profiles by using parallel quantitative PCR array targeting 296 genes. Samples were collected from different locations in Finland: manure from cattle and pig farms, soil that received the manure as fertilizer, sediments from aquaculture farms and effluent, influent and activated sludge from waste water treatment plant. Total DNA was isolated and the genes were quantified using highly parallel qPCR array on Applied Biosystems OpenArray platform and normalized with 16S rRNA gene numbers. Altogether 184 genes were found from the waste water treatment plant. Untreated inflow water contained the highest number of genes, followed by the final effluent and sludge. 71 of the genes were found in aquaculture sediments. The antibiotic resistance genes against to the antibiotics that have been used in the aquaculture farms were clearly enriched. The numbers of the antibiotic resistance genes was increased in the cattle and pig manure in the farms that used antibiotics and the spreading of the manure increase that amount of antibiotic resistance genes in the soil. In addition to the antibiotic resistance genes, the genes related to the genetic mobility were enriched by human impact which may implicate increase in the horizontal gene transfer by human activities. Our results demonstrate that human activities can results to the increase to the abundance of antibiotic resistance genes which may lead to the enrichment of the resistance and transfer of the resistance genes to the pathogens.

Passive Sampling in the Aquatic Environment: Recent Developments and Advances

RP156 Evaluation of porewater reductions due to carbon placement via sedimite and aquagate at a contaminated sediment site

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Activated carbon is increasingly used to reduce contaminant bioavailability in sediments as a remedial measure. Activated carbon is often placed as a composite material to aid in settling and retention at the sediment surface. A demonstration of two such composite materials, Sedimite and Aquagate, was conducted in PCB contaminated sediments in open water near Hunters Point, CA. The materials were allowed to settle directly to the sediment surface. Here the changes in porewater concentration in the surface sediments and in deeper layers up to 40 cm below the sediment surface were evaluated by passive sampling using polydimethylsiloxane (PDMS) coated fibers to measure porewater concentrations of PCBs. Our goal was to evaluate and compare changes to in situ hydrophobic organic compound (HOC) porewater concentrations before and after activated carbon placement and between the two different activated carbon placement methods. The pilot study was conducted at Hunters Point Naval Shipyard (HPNS). For passive sampling, SPME fibers (34.5 µm PDMS coating) were preloaded with 7 C^{13} PCB congeners as performance reference compounds (PRCs). PDMS fibers were inserted to unshielded holders, attached to a tripod frame and embedded vertically 30 cm into the sediment for triplicate measurement of porewater concentration at the 20 sampling locations. After 28 days the fibers were retrieved, sectioned into a 1-6, 11-16 and 21-26 cm segments below the sediment surface and analyzed for PRCs and 111 PCB congeners using GCTQMS. Bulk sediment samples were also collected and the concentrations quantified by GCTQMS. Baseline sampling showed uniform porewater concentrations across the site with lower concentrations in the near surface versus the deeper layers due to exchange with the overlying water from the shallower zone. Statistical analysis showed that the porewater samples were well correlated with nearby samples. After AC placement, porewater concentrations decreased from a baseline average of 2.2 ng/L to 0.35 ng/L in the surficial layer. Smaller but significant reductions in porewater concentration was noted at the deeper depths. AC also influenced the approach to steady state of the passive samplers with more rapid approach to steady state post-placement. The influence of nonlinear sorption on the PRC analysis was evaluated by modeling and indicated that the presence of activated carbon can cause traditional analyses to overestimate the fractional approach to steady state.

RP157 In situ porewater sampling of PCDD/Fs and PCBs to predict their bioaccumulation in Passaic River benthic invertebrates

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Polyethylene samplers were deployed four times at four different locations along the lower Passaic River in sediment and water column from June, 2015 to February, 2016. At the same time, sediment and benthhic invertebrates were collected from the deployment sites. All samples were analyzed for mono- through octa-chlorinated dioxins and furans, and polychlorinated biphenyls, PCBs. Concentrations of sum 89 PCBs in the porewater as obtained from the in situ sampler ranged from 2.0 ng/L (site S1) to 5.0 ng/L (site S2) with an average concentration of 3.0 ng/L. PCDD/F concentrations in porewater generally ranged from 24 pg/L (Site 1) to 41 pg/L (Site 3) with an average concentration of 32 pg/L. In both cases, in situ porewater concentrations were in the same range as laboratory eqiulibrations of those sediments with passive samplers. For the in situ eqiulibrations, the use of PRCs was of critical importnace to correct for non-eqiulibrium attained during field deployments.Three different PRC combinations were compared to laboratory-obtained porewater results. For the in situ multisampler deployments, there is some benefit of using both d-PAHs + $^{13}C_{12}$-PCDDs for PCDD/Fs quantitation. Freely dissolved concentrations of PCBs in the river water ranged from 1.3 ng/L to 1.8 ng/L, with no statistical significant difference between the different sampling sites. Lipid normalized concentrations of PCBs and PCDD/Fs were estimated from sediment, porewater and river water and compared to measured lipid normalized concentrations in the benthic species. Porewater and sediments using the black and organic carbon scenario were generally better predictors of lipid concentrations of PCBs and PCDD/Fs. Additionally, good prediction of the lower chlorinated PCBs (mono- through tetra-) were also obtained from the river water (polyethylene deployed in the water column above the bottom sediments). Based on our results, exposure to porewater and to a lesser extent river water and the probable ingestion of sediment particles are the possible sources of bioaccumulation of PCBs and PCDD/Fs in the lower Passaic River, which indicates that deployed olyethylene samplers (either in the river water or the sediments) are good predictors of tissue concentrations of the benthic species.

RP158 Passive samplers for in situ measurement of pyrethroid insecticides in surface water

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Pyrethroid insecticides are widely used in urban environments, and their occurrence in urban surface streams has been associated with acute aquatic toxicity to invertebrates, especially in regions such as California. Pyrethroids are strongly hydrophobic compounds, and studies have shown that the freely dissolved concentration (C_{free}) is more predictive of their bioavailability and toxicity to aquatic species. In this study, we developed a passive sampling approach for in situ monitoring of low levels of pyrethroid insecticides under field conditions. Evaluation of a series of polymers suggested that polyethylene film (PE) exhibited the highest uptake capacity for pyrethroids. To overcome the slow uptake kinetics and circumvent the requirement of long time interval to reach equilibrium, isotope-labeled permethrin and bifenthrin were used as performance reference compounds (PRC) to preload the PE sampler. Desorption of isotope-labeled pyrethroids and absorption of non-labeled analytes were isotropic, validating the assumption for the PRC method. The PRC-PE calibration method was then validated in large tanks under simulated flow conditions, where the effect of salinity and dissolved organic matter on bioavailability was also determined. The optimized method was further deployed for 7 d at multiple locations in southern California for in situ monitoring of pyrethroids. The results indicated that the PRC-PE sampler may be used for in situ monitoring of pyrethroids in surface streams under field conditions with flexible sampling intervals and sufficient sensitivity.

RP159 Using a Commercially-available Passive Sampling Device To Monitor Hydrophobic Organic Contaminants in the Laboratory and the Field

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Passive sampling devices (PSDs) present many advantages over conventional aqueous samples for quantifying hydrophobic organic compound (HOCs) concentrations in pore water (sediment and soil), surface water and storm water in terms of cost and data usability. They provide data to estimate contaminant bioavailability and toxicity to environmental receptors that is more representative than conventional grab samples, as it quantifies contaminants only in the dissolved form. In the field, PSDs enable collection of representative- and depth-discrete data without the need to collect large volume aqueous samples. Similarly, in laboratory treatability studies to evaluate remedial options for contaminated sediments or soils, passive samplers help to decrease the volume and number of samples required. Passive samplers deployed in these studies provide the ability to compare different treatment technologies in a relatively short time frame, test multiple conditions concurrently, and enable the flexibility to allow changes to a remediation strategy that would be impractical at field-scale. This presentation will highlight the benefits of using PSDs in both laboratory treatability studies and field applications as well as discuss the practical lessons learned from applying passive sampling techniques, drawing from several case studies using a commercially-available PSD. In one study, PCB availability of a sediment amended with activated carbon, zero valent iron, siderite and sulfur was measured with PSDs. The results were used to determine the most effective commercial amendment for decreasing PCB concentrations in pore water, with PSDs demonstrating that the selected remedy achieved a >95% reduction in PCB availability. In another study, PSDs were used to characterize PCB concentrations in sediment pore water amended with different amounts of activated carbon and the results were used to determine the optimal dose of carbon for the field application, with PSDs demonstrating that the optimal activated carbon application rate resulted in a 98% reduction in PCB availability. At this same site, PSDs were deployed in the field to provide baseline PCB availability data prior to activated carbon amendment, and the PSDs will be used for post amendment monitoring to document remedial success. It is clear that currently-available passive sampling approaches are ready for widespread application and can provide high-quality data to aid environmental decision makers.

RP160 Sensing organic pollutants in aquatic environment by passive sampling techniques

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Organic contaminants, such as persistent organic pollutants, have the potential to cause adverse effects on humans and wildlife because of their toxicity and bioaccumulation and/or biomagnification through the aquatic foodweb. Measurement of dissolved organic contaminants in water can provide important information for predication of organic contaminants in aquatic species, as well as the magnitude of human exposure. Passive sampling techniques are easy to operate and cost-effective, hence create new opportunities for monitoring organic contaminants in aquatic environment around the world. Among the available passive samplers, polyethylene (PE) devices have been recommended as preferred alternatives for sensing organic contaminants in aquatic environment because they are biomimetic, inexpensive, and convenient for field deployment. A self-developed passive sampler with PE as sorbent phase has been demonstrated to be capable of reasonably quantifying dissolved organic contaminants in coastal seawater and in freshwater lakes in Antarctica and China. The highest level of PAHs in Antarctica and in China was found around Russian Progress II Station and Southern Lake in Wuhan, respectively, both indicating the significance of human activities to the loadings of PAHs. In China, the concentrations of PAHs gradually increased from Western China (Xinjiang and Tibet) to Eastern China (Liaohe River, Songhua River and the lower reaches of the Yangtze River), showing a similar strong correlation with population density. The composition profiles of PAHs in Antarctica indicated that PAHs in the inland lakes were derived mainly from local oil spills, while mixed PAH sources, i.e., coal combustion, refined fossil fuel combustion and oil spills, occurred in China. Our results indicate the feasibility of establishing a global aquatic network of organic contaminants with PE passive samplers, which is critical for enhancing the long-term health risk assessment for humans and wildlife.

RP161 Passive sampling for priority substances at semi polluted River Porvoonjoki, Finland

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European Union has set the water policy directive 2008/105/EC which classifies 45 substances or substance groups as priority substance list. It sets environmental quality norms for annual average and maximum allowable concentration for inland and other surface waters and biota. From a monitoring perspective simple and reliable method is of major importance. In this study the performance of specific passive sampling device for the monitoring of dissolved concentrations of three priority substances was assessed through a field deployment of samplers along slightly polluted river in Southern Finland. Selected substances are commonly used in Finland for different purposes. Polybrominated diphenyl ethers (PBDE) are used as flame retardants. Neurotoxic cypermethrin is a pesticide that belongs to pyrethroids. Nonylphenol is used by chemical industry and is endocrine disruptor. They are all in the priority substance list and are considered toxic. They are all also lipophilic (log K_{ow} >3), and therefore they should accumulate in lipid passive samplers. River Porvoonjoki is 143 km long and its drainage basin is 1273 km^2. The river is located in Southern Finland and runs from the Lahti region to the Gulf of Finland. Three wastewater treatment plants along the river are the major source of chemical input to natural watercourse including a discharge of treated wastewaters of 150 000 citizens. The natural flow of the river is low and from time to time one third of the river's water can be treated wastewater. Passive sampling devices accumulate chemicals continuously from water providing a measure of average river conditions and pollution status over the exposure period of 30 days instead of active water sampling that only gives information of exact moment. The sampling was taken with passive sampling devices (PSDs) from water. The PSDs were 48 cm long low-density polyethylene (LDPE) layflat tubing filled with 0.5 mL of triolein. Specific sampler type was chosen because the chosen chemicals have high log K_{ow}, thus highly lipophilic, and are uptaken by triolein. The samples were taken from water because the EU's WPD gives quality norms for waters. The samplers were deployed to the river Porvoonjoki on September 2015 and the study was repeated on summer 2016. The chemicals were analysed with GC-MS. Uptake rates of the chemicals were either taken from the literature (nonylphenol and PBDE) or uptake rates were determined at laboratory (cypermethrin).

RP162 Field evaluation of membrane assisted passive sampler for isolation and identification of pharmaceutical and personal care products in natural waters

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One of the major challenges in routine monitoring programmes is the isolation and identification of substances whose fate, behaviour and (eco) toxicological effects are not well understood. Pharmaceuticals and personal care products and their degradation products exhibit adverse effects on biota and human health, yet their occurrence, distribution and fate is not well understood. This work reports findings on the use of passive sampling devices in addressing the challenge of isolation and identification of selected emerging pharmaceuticals and personal care products and their degradation products in natural waters. Aqueous sampling rates for selected pharmaceuticals and personal care products were determined on the membrane assisted passive sampling device (MAPS) and commercially available Polar Organic Chemical Integrative Sampler (POCIS). Field calibration studies of the passive sampling devices spiked with performance reference compounds were performed, and the resulting time-weighted average concentration estimates were compared with laboratory derived values.

RP163 Fugacity gradients of hydrophobic organics across the air-water interface measured with a novel passive sampler

W. Chen Chou; L. Bao, E.Y. Zeng, Jinan Univ / School of Environment

Mass transfer of hydrophobic organic contaminants (HOCs) across the air-water interface is an important geochemical process controlling the fate and transport of HOCs at the regional and global scales. However, few studies have characterized concentration or fugacity profiles of HOCs near both sides of the air-water interface, which is the driving force for the inter-compartmental mass transfer of HOCs. Herein, we introduce a novel passive sampling device which is capable of measuring concentration (and therefore fugacity) gradients of HOCs across the air-water interface. Laboratory studies indicated that the escaping fugacity values of polycyclic aromatic hydrocarbons (PAHs) from water to air were negatively correlated to their volatilization half-lives. Results for field deployment were consistent between the passive sampler and an active method, i.e., a combination of grab sampling and liquid-liquid extraction. In general, the fugacity profiles of detected PAHs were indicative of an accumulation mechanism in surface microlayer in the study regions (Haizhu Lake and Hailing Bay of Guangdong Province, China), while p,p'-DDD tended to volatilize from water to the atmosphere in Hailing Bay. Furthermore, the fugacity profiles of the target analytes generally decreased and then increased towards the air-water interface, reflecting the complexity of environmental behavior of the target analytes near the air-water interface. Overall, the passive sampling device provides a novel means to better characterize the air-water diffusive transfer of HOCs, facilitating the understanding of the global cycling of HOCs.

RP164 Temporal and spatial monitoring of persistent organic pollutants on the Palos Verdes Shelf using two passive sampling methods

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Passive sampling methods now allow for the collection of large amounts of data with minimal effort. Because large data sets are necessary to determine spatial and temporal trends in the concentrations of persistent organic pollutants (POPs) in the coastal ocean, passive sampling tools are ideally suited to these types of studies. In September 2010 and 2013, polyethylene films (PE) and solid-phase microextraction fibers (SPME) were co-deployed to determine the dissolved concentrations of POPs on the Palos Verdes Shelf (PVS) Superfund site. During the 2010 sampling event, the two types of samplers produced similar but offset results. The goals of the 2013 sampling campaign were to (1) investigate the potential sources of the offset between results from the two sampler types, and (2) investigate the temporal and spatial stability of concentrations at the Superfund site. Results from both sampling campaigns indicated increasing dissolved concentrations of POPs with proximity to sediment bed, and in a north-west direction along the shelf for several km before the concentrations began to decrease. Temporally, concentrations between the two sampling events were within a factor of three. Additionally, the use of temperature correction factors to PE and the use of performance reference compounds for SPME impacted the concentrations by factors of two and three, respectively. Despite two successful congruent sampling events, additional monitoring will be needed to determine long-term trends or statistically significant changes in dissolved POPs on the PVS.

RP165 Reducing the Impact of Flow on the Sampling Rate of POCIS

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Polar Organic Chemical Integrative Samplers (POCIS) have been demonstrated as a promising technique for measuring water concentration of many organic compounds. However, current sampler designs and technology do not account for variations in sampling rate due to changes in flow velocity and turbulences near the sampler. Thus, our research targeted two different approaches to improve calibration of samplers including: 1) placement of flow restrictive screens around the sampler to cause a stable boundary layer to develop around the sampler; and 2) test potential performance reference compounds (PRCs) to measure in-situ sampling kinetics allowing adjustments of sampling rates. Moderately high flow (10 cm/s) resulted in 466 and 418 % higher sampling rates for TNT and atrazine, respectively, with no modification to the POCIS. However, placement of a nylon mesh screen (0.100 mm mesh) on the outside of the POCIS reduced differences in sampling rates across flow to less than 25%. In a separate experiment, stable isotope labelled caffeine was used as a PRC. Through correction of the sampling rate based on the rate of loss of caffeine from each sampler, differences in sampling rates across flow were reduced to less than 30% for TNT and atrazine. However, the PRC did not perform as well for all analytes tested. Both techniques are promising for increasing the accuracy of POCIS.

RP166 Calibration of Organic-Diffusive Gradients in Thin Film (o-DGT) Passive Samplers for Perfluorinated Chemicals (PFCs) in Water

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The calibration of perfluorinated compounds (PFCs) was assessed with the organic-diffusive gradients in thin films (o-DGT) passive sampler. Monitoring for PFC contaminants in waters is important given their environmental persistence and bioaccumulation. The few attempts at passive sampling for PFCs have largely relied on the polar organic chemical integrative sampler (POCIS). While successful, determination of chemical concentrations with POCIS can be plagued by significant uncertainties given limitations in predicting effects of environmental variables (e.g., water flow rate, temperature, etc.) on POCIS sampling rates. The main advantage of o-DGT is its insensitivity in sampling rate towards flow rate of water, eliminating the need for extensive in situ calibration. Six PFCs were studied: perfluoro-n-decanoic acid, perfluoro-n-nonanoic acid, perfluoro-n-undecanoic acid, perfluoro-n-octanoic acid, perfluoro-1-octanesulfornic acid, and perfluorobutane-1-sulfonic acid. A diffusive cell experiment showed that diffusion coefficients of these PFCs through the diffusive gel layer ranged from 2.20 to 3.88×10^{-6} cm^2/s at 23°C. A calibration test was performed to determine the kinetic uptake of PFCs to o-DGT; these experimental sampling rates were compared to theoretical values (i.e. Hayduk-Laudie model) and to the measured diffusion coefficients. The sampling rates calculated from measured diffusion coefficients were in general agreement with the sampling rates measured in the calibration experiment (relative errors < 40%). This result indicates that diffusion coefficients alone can estimate sampling rates for o-DGT, thus eliminating the need for full scale laboratory calibration required in POCIS.

RP167 Extending the scope of passive sampling for contaminated sediments: standardizing methods for moderately hydrophobic organic contaminants

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Passive sampling methods for determining the freely dissolved concentrations (Cfree) of organic contaminants in sediment are focused on those that are highly hydrophobic (e.g. log Kow > 5). However, organic contaminants of concern are not limited to only those that meet this criteria. In this study, we propose standardizing and validating protocols that utilize different polymeric materials to target chemicals of concern in sediment that exhibit a wide range of hydrophobicity (i.e. 3

RP168 Accessibility of Diffusive gradient in thin films (DGT) Samplers to representative Hg forms in sediments

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Interstitial or sediment pore water concentrations have been demonstrated to relate with bioavailability and bioaccumulation. However, traditional techniques used for pore-water sampling such as the Henry's sampler have severe limitations (e.g., inability to obtain uncompromised sample at multiple depth intervals) resulting in inadequate sediment characterization. Recent development and application of passive sampling devices such as diffusive gradient in thin film (DGT) samplers has mitigated many of the addressed issues. DGTs can profile in-situ Hg pore-water in sediments at relatively high resolution and at time scales relevant to biological processing. Despite its advantages, the question still remains on what DGT really measures and how it relates to the 'truly' bioavailable mercury fraction in sediment pore-waters. Particulate Hg (> 0.45 µm) is neither expected to be bioavailable nor accessible by DGTs, whereas, the truly dissolved (< 1 nm) are readily accessible by both biota and DGT. The bioavailability of intermediate sizes is unknown but colloidal particles > 20-50 nm in size are unlikely to pass a cell wall without active transport processes. Similarly, colloidal particles in this size range have diffusivities 10-100 times are unlikely to diffuse into a DGT at significant rates. To explore this hypothesis, different molecular sizes of Hg inorganic/organic complexes (from 'dissolved' to high molecular weight colloids) were prepared followed by measurement of the Hg uptake by DGTs. The Hg-organic colloids included both broadly size fractioned natural organic matter and distinctly sized synthetic polymers containing strong Hg binding sites. The measured diffusion coefficient of these colloidal-Hg forms through the DGT gel layer were then correlated to the size fractions accessible by the DGTs. Finally, the results from these studies will be used to discuss the biological relevance (e.g., methylation potential) of different Hg size forms taken up by DGTs.

RP169 Polyethylene: An Alternative Passive Sampler for Monitoring Fluorotelomer Alcohols

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Fluorotelomer alcohols (FTOHs) and other poly-fluorinated alkyl substances (PFASs) are common and ubiquitous by-products of various industrial telomerization processes. This class of volatile and semi-volatile compounds has been shown to degrade into a wide variety of perfluorinated carboxylic acids (PFCAs) including perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS), persistent organic pollutants. Recent atmospheric studies have shown the presence of fluorotelomer alcohols and their degradation products present in high concentrations spreading out from point sources in North America, Europe, and Asia. This study develops a method for the widespread monitoring of fluorotelomer alcohols in air and water through the use of polyethylene (PE) passive samplers and gas chromatography-mass spectrometry (GC/MS). To achieve this, PE-water and PE-air partitioning coefficients need to be known. Kpe-w's were determined via a time series of water-PE exposures using known concentrations of PFASs including FTOHs, sulfonamides (FOSAs), and sulfonamidoethanols (FOSEs). A field validation study in Providence was set up to determine the Kpe-a's via a time series of exposure. Log Kpe-w's, for FTOHs were found to range from 2.8-5.3 while sulfonamides (FOSAs) and sulfonamide ethanols (FOSEs) were found to range from 4.9 to 5.4.

RP170 Field Evaluation of organic-DGT and POCIS: Presence and Sources of Pesticides and Pharmaceuticals in the Lower Red River

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Current passive sampling designs for polar organic contaminants in water lack the ability to easily adjust uptake rates under varying environmental conditions (e.g., flow rate, temperature), often leading to large uncertainties in measurements. This issue significantly reduces the overall applicability of polar passive samplers (e.g., Polar Organic Chemical Integrative Samplers; POCIS). The diffusive gradients in thin films (DGT) sampler, popular for metals sampling, is largely insensitive to effects of flow on sampler uptake and allows for simple measurement/modelling of temperature-specific sampling rates. Our previous work successfully adapted and calibrated DGT samplers for a suite of polar organic contaminants (hence, organic-DGT), demonstrating that uptake by o-DGT was linear over 3-4 weeks at environmentally relevant concentrations, and compound specific sampling rates were largely independent of water flow. We evaluated the utility of the o-DGT passive sampler in the Red River, a large river system originating in the United States and emptying into Lake Winnipeg, Canada, the 10th largest freshwater lake in the world. A total of six sampling sites along the Red River were monitored continuously from April to September (2-3 week deployments) to assess transport of pesticides and pharmaceuticals from the US-Canada border into Lake Winnipeg. To validate o-DGT, samplers were co-deployed with POCIS to highlight the benefits and limitations of both. The herbicide atrazine was detected at all sites over the entire season, with concentrations ranging from 10-30 ng/L. Neonicotinoid insecticides, including thiamethoxam and clothianidin were only detected at the agricultural dominated sites where concentrations from 5-50 ng/L were observed. Various pharmaceuticals, including trimethoprim, sulfamethoxazole, and carbamazepine, were generally detected only downstream of the city of Winnipeg at concentrations up to 15 ng/L. In most cases o-DGT and POCIS were in general agreement, however at certain sites and times POCIS appeared to systematically underestimate water concentrations compared to o-DGT, likely a result of POCIS uptake being highly variable with water flow and temperature. This study illustrates the promise of o-DGT as a robust, widely applicable monitoring tool with the ability to account for flow-rate and temperature effects in-situ, two factors plaguing the reliability of current polar passive samplers.

RP171 o-DGT passive sampler for in situ sampling of antibiotics in waters

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Passive water sampling has several advantages over active methods: providing TWA data, saving time and cost and yielding high spatial resolution data. One problem of many current samplers is that their sampling rates for are flow-rate dependent, thereby requiring calibration data and other information to enable water concentrations to be derived. A novel passive sampler (called o-DGT) for organic chemicals (antibiotics as model compounds) based on diffusive gradients in thin-films (DGT) was developed[1] and tested[2] to overcome some drawbacks of current samplers, and then employed to assess the removal of antibiotics in WWTPs[3]. DGT can be extended to organic chemicals with XAD18 as the new binding phase and agarose as the novel diffusion gel. The measured diffusion coefficient (D) with a diffusion cell allows temperature correction and derivation of aqueous concentrations. o-DGT sampler was then tested in a waste water treatment plant (WWTP) for a range of antibiotics. Fourteen antibiotics were detected in the actively sampled water samples, with 10 of the 14 detected in o-DGT deployed for more than 7 days. This time interval is recommended to integrate aqueous concentrations over time, without risks of reaching capacity and significant biofouling. DBL thickness had less effect on the o-DGT measurement than reported for other passive samplers. o-DGT gave comparable results to the automatic sampling but is more cost effective; both were more satisfactory than grab sampling. Sampling in two WWTPs (Chinese and British) showed that Neither of the WWTPs was very effective at removing antibiotics: ~40–50% (overall) was removed by the two plants, with the rest being discharged into the receiving rivers. o-DGT is useful and cost-effective tool for routine environmental monitoring and can highlight the effectiveness of treatment steps, which can be applied to wastewater based epidemiology studies.

RP172 Development of a novel passive sampling strategy for methylmercury in sediments and soils

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Mercury in sediments can pose a significant risk to ecological and human health when it is converted to the more toxic and bioaccumulative methylmercury (MeHg) by anaerobic microorganisms near the sediment-water interface. Benthic organisms exposed to MeHg in this zone represent an important link in the process of MeHg bioaccumulation in aquatic food webs. As such, quantitative measures of the bioavailability of MeHg to benthos are important for contaminated site risk assessment. To date, no passive sampling strategy has achieved wide acceptance for generating such measurements. Existing equilibrium technologies such as "peepers" are based on size exclusion, while the diffusive gradient in a thin film (DGT) approach employs high-affinity chemisorption and operates in a continuous, kinetic mode. We are working to develop a novel passive sampling technology for MeHg using a hybrid accumulation strategy to emulate the steady-state (pseudo-equilibrium) mode of bioaccumulation by benthos. A target for sampler partitioning of MeHg was established at log K between 3.0 and 4.5, to approximate typical benthic bioaccumulation factors and sediment-water partitioning constants. A variety of custom materials consisting of either thiolated sorbents or activated carbon embedded in support polymers was prepared and evaluated in increasingly environmentally realistic experiments. In initial tests in 3 ppt Instant Ocean solution, many of the materials showed strong, linear partitioning of MeHgCl across a relevant range of concentrations. Subsequent tests in the presence of MeHg complexed to dissolved organic matter found somewhat decreased accumulation by polymers, but log Ks remained in or near our target range. The most promising polymers were exposed in contaminated soil slurries. Polymer log K values were within 0.5 log units of soil K_d. Pore water MeHg concentrations estimated using polymer accumulation and measured partitioning constants for the MeHg-DOM complex agreed within a factor of one to four with direct measurements of centrifuged pore water. An experiment is now underway to characterize the kinetics of uptake by polymers. Future work will attempt to correlate MeHg bioaccumulation by the amphipod L. plumulosus with measurements by our sampling polymers deployed alongside the organisms in test microcosms. Sampling measurements will be used as input for a bioaccumulation model to validate the predictive capability of the device.

RP173 Next-generation environmental passive sampler materials: Electrospun nanofiber mats (ENMs)

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Using electrospinning, we synthetized seven electrospun nanofiber mats (ENMs) from polymers (ethylene-vinyl-acetate, polyacrylonitrile, polyethylene terephthalate, polymethylmethacrylate, polystyrene, polyvinyl acetate and polyvinylidene fluoride) and investigated their performance as next-generation passive sampling materials. Given the ease of material fabrication, we were able to tune the physical-chemical properties of ENMs to produce robust materials with improved selectivity and enhanced sorption capacities for polar and nonpolar pollutants. We tested ENM in homogeneous aqueous systems and heterogeneous systems (model soils) against aniline and nitrobenzene, and PCBs and dioxin. Aqueous uptake experiments yielded very fast rates of partitioning with equilibration times < 1 d. Equilibrium partition coefficients (L/kg) for ENMs ranged from 0.7 to 3 log units for aniline and nitrobenzene, suggesting uptake via partitioning into the bulk nanofiber (i.e., absorption) and specific binding interactions (e.g., H-bonding, Coulombic interactions). PCBs and dioxin yielded very fast equilibrium uptake (< 18 h), with equilibrium partition coefficients ranging from 3 to 6 log units. The partition coefficients measured for the best performing ENMs often exceeded partition coefficients achieved with available passive sampling materials (e.g., LDPE, PDMS glass fiber). Across a range of experimental conditions (pHs, analyte concentration and mixtures), little change in ENM performance was observed. We also found promising performance in heterogeneous systems with model soils, where polystyrene, not only yielded reproducible measurement of nitrobenzene pore water concentration but also allowed ease of handling by minimizing unwanted ENM-soil organic matter interactions. Further efforts improved performance via fabrication of novel ENM composites and surface-chemical functionalized ENMs. The inclusion of carbon nanotubes or anionic surfactants could be used to promote uptake of aniline (by as much as 1 log unit in partition coefficient). Further, integration of Ag nanoparticles to ENMs can be used to impart biocidal activity, thus slowing biofouling during deployment. We also fabricated novel multilayer ENMs, in which layer-by-layer combinations of different polymers impart multi-target capabilities (e.g., simultaneous uptake of polar and nonpolar species) and greater ease of application and handling in complex media (e.g., protective surface layers that limit fouling).

RP174 Bioanalytical Effect-Balance Model to Determine the Bioavailability of Organic Contaminants in Sediments Affected By Black and Natural Carbon

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Several important binding entities in sediments dictate the fate and availability of organic contaminants in marine sediment water systems. Black carbon (BC) has been shown to have a high sorptive capacity for organic contaminates and to limit their bioavailability and thus the risk of exposure to organisms, while the fraction bound to organic carbon (OC) is considered to readily desorb and thus this fraction is bioavailable. So far, binding of single compounds or defined compound classes to BC in natural sediments has been investigated using chemical analysis. In this study we expand the investigation to mixtures of chemicals by combining passive sampling with in vitro bioanalytical tools. The advantage is that these tools will not only give information on the bioavailability of sediment-associated contaminants but also their mixture toxicity. As a case study we investigated sediments from an Australian coal-exporting harbour with high BC load, and sediments from a tropical agricultural area and a subtropical urban area with lower BC contents as reference sites. Accelerated solvent extraction was used to exhaustively extract sediments. Tenax extraction and PDMS (polydimethylsiloxane) passive sampling were used to differentiate total from readily desorbable (bioavailable) concentration, and overlaying water was extracted with solid phase extraction and served as a proxy for pore water. All extracts were characterised with two cell-based bioassays, the AhR-CAFLUX, which measures dioxin-like activity, and the AREc32 bioassay, which quantifies the induction of the adaptive stress response to oxidative stress. The resulting bioanalytical equivalents, which are effect-scaled concentrations, were applied in an effect-balance model, consistent with a mass balance-partitioning model for single chemicals. Sediments containing BC had most of the bioactivity associated with the BC fraction, while the OC fraction played a role for sediments with lower BC. As effect-based sediment-water distribution ratios demonstrated, most of the bioactivity in the AhR-CAFLUX was attributable to hydrophobic chemicals, while more hydrophilic chemicals activated AREc32, even though bioanalytical equivalents in the aqueous phase remained negligible. This approach can be used to understand the fate and effects of mixtures of diverse chemicals in sediments and make informed risk based decisions concerning the fate of contaminated sediments.

RP175 Evaluating the Relationship between Equilibrium Passive Sampler Uptake and Aquatic Organism Bioaccumulation

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This review evaluates passive sampler uptake of hydrophobic organic contaminants (HOCs) in water column and interstitial water exposures as a surrogate for organism bioaccumulation. Fifty-four studies were found where both passive sampler uptake and organism bioaccumulation were measured and 19 of these investigations provided direct comparisons relating passive sampler uptake and organism bioaccumulation. Polymers compared included low density polyethylene (LDPE), polyoxymethylene (POM), and polydimethylsiloxane (PDMS), and organisms ranged from polychaetes and oligochaetes to bivalves, aquatic insects, and gastropods. Regression equations correlating bioaccumulation (C_L) and passive sampler uptake (C_{PS}) were used to assess the strength of observed relationships. Passive sampling based concentrations resulted in logarithmic predictive relationships, most of which were within one to two orders of magnitude of measured bioaccumulation. Mean coefficients of determination (r^2) for LDPE, PDMS and POM were 0.68, 0.76 and 0.58, respectively. For the available raw data, the mean ratio of C_L and C_{PS} was 10.8 ± 18.4 (n = 609). This review concludes that in many applications passive sampling may serve as a reliable surrogate for biomonitoring organisms when biomonitoring organisms are not available. When applied properly, passive sampling based estimates of bioaccumulation provide useful information for making informed decisions about the bioavailability of HOCs in aquatic environments.

RP176 A Novel Approach to Assess and Quantify Mass Flux of Groundwater Discharge into Surface Water

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Discharge of contaminated groundwater to surface water occurs at many sites adjacent to water bodies. Due to a lack of cost-effective methods to quantify mass-flux, cleanup objectives for groundwater are typically very conservative resulting in unnecessary treatment costs. There is a need for cost effective and accurate hyporehiec zone characterization tools to better estimate contaminant level impacts, verify natural attenuation, and measure groundwater mass-flux over broader space and time dimensions. With support from CH2M, the University of Florida (UF) has developed a sediment-bed passive flux meter (SBPFM) to address these needs. The SBPFM in an evolution of UF's Passive Flux Meter (PFM) that is well established in the remediation field. The main design challenge in developing the SBPFM was converting the PFM, which measures horizontal groundwater flux, to a vertical configuration for hyporhiec and groundwater/surface water transition zone application. UF developed the SBPFM through lab bench-scale testingthat has assessed its design efficacy in measuring contaminant flux. The SBPFM was designed to passively and directly measure local contaminant and water fluxes and provide more accurate information on the temporal mass flux distribution through the sediments in order to better design site remedial and closure strategies. Once lab tests of the SBPFM were completed, field tests were conducted by CH2M and UF at several sites. The field deployments were designed to both refine the logistics of deployment processes through optimal device configurations, and obtain data that could identify groundwater flux (detection of contaminants and estimates of mass flux). The field deployments showed that the SBPFMs could be easily deployed near shore and provide results for both tidal and non-tidal conditions. Additionally, both activated carbon and ion-exchange resins were used to measure organic (VOCs) and inorganic (chloride) contaminants, respectively. The deployment results demonstrate that reliable measurements could be achieved since time-averaged flux was estimated as opposed to short-term or "point-in-time" measurements. An economic assessment of a SBPFM deployment relative to other sediment flux tools also demonstrates potentially significant cost savings.

Deepwater Horizon Oil Spill – The Discoveries and Outreach

RP177 Characterization of Dioctyl Sodium Sulfosuccinate as an Obesogen In Vivo

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Childhood, adolescent and adult obesity rates in the United States and worldwide have been increasing since the 1970s and have reached epidemic proportions. While genetic predisposition, diet and exercise are known factors in obesity development, recent studies have focused on elucidating environmental exposures associated with obesity.
Our specific focus is on exposures to endocrine/metabolic disrupting chemicals known as obesogens, which can increase stem cell to fat cell differentiation, and have other metabolic effects that collectively promote obesity development. Recently, our lab identified dioctyl sodium sulfosuccinate (DOSS) as a PPARg agonist and a probable obesogen and wanted to further validate its obesogenic potential in vivo using dosing and exposure routes common to human scenarios. DOSS is a commonly used non-ionic surfactant, present in Corexit dispersants that were used during the Deepwater Horizon oil spill, and is also present in a variety of consumable and personal care products as an emulsifying agent. Pregnant women often become constipated at midgestation and are prescribed as the standard of care stool softeners that are essentially DOSS (500mg/day) through nursing. To parallel this scenario, pregnant female mice were orally dosed with either 30ug/mL DOSS in carboxy methylcellulose (0.5% CMC) vehicle or vehicle control alone in their drinking water from midgestation (day E11.5) through weaning. F1 offspring were then given untreated water until they were 16 weeks old and end point analysis for obesity markers was performed including glucose tolerance, DEXA scans for fat content and bone density, liver and fat pad histology and gene expression and bone marrow mesenchymal stem cell gene expression and differentiation potential. Our results indicate that DOSS is a bona fide obesogen in vivo and displays a sexually dimorphic phenotype. Most significantly, we observe that DOSS exposed males had a significantly higher fat percentage and significantly lower bone area when compared to vehicle treated controls. Additionally, treated males responded different to the glucose tolerance test, displaying a significantly higher spike in glucose blood level after glucose gavage despite similar baseline levels. Cellular and molecular changes were also observed that are indicative of DOSS' obesogenic potential in vivo. Together, these data suggest that DOSS can act as an obesogen in vivo at physiological levels of exposure.

RP178 Identifying and characterizing Deepwater Horizon oil's toxic effects across taxa

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As part of the Deepwater Horizon Natural Resource Damage Assessment (NRDA), the Natural Resource Trustees conducted a comprehensive laboratory toxicity testing program with fish, zooplankton, phytoplankton, amphipods, mollusks, crustaceans, birds, turtles, and mammalian cell lines. Across tests, the Trustees found a considerable degree of consistency among types of toxic responses observed in different organisms. Cardiotoxicity, poor reproduction, abnormal growth/development, disruption of blood cells and function, oxidative damage, immune system dysfunction, impairment of stress responses and adrenal function, and effects on locomotion were observed across a variety of taxa. This consistency points to the conservation of mechanisms of action and disease pathways across a range of vertebrate and invertebrate species. Results from field studies on wildlife from habitats contaminated with DWH oil demonstrate similar manifestations of toxic effects. From a toxicological perspective, we observed a logical progression of physiological perturbation from molecular and cellular effects that manifest as organ disease, to systemic effects that compromise fitness, growth, reproductive potential, and survival. From a clinical perspective, the breadth of adverse health effects and symptoms associated with exposure to Deepwater Horizon oil forms a coherent suite of symptomatic responses to petroleum toxicity. The availability of data from the large number of laboratory and field studies focused on DWH oil toxicity provides an unprecedented opportunity for scientists to investigate the mechanisms behind oil toxicity, and for decision makers and the public to gain a broader, ecosystem wide understanding of how oil spills affect natural resources.

RP179 The impact of crude oil exposure on the prey capture and male aggression behavior in sheepshead-minnow (Cyprinodon veriegatus veriegatus)

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From April to July 2010, the Deepwater Horizon oil spill released 3.19 million barrels of crude oil into the Gulf of Mexico that then spread to the surrounding tributaries and wetland habitats. In an attempt to reduce the effects of an oil slick on coastal habitat, dispersant was applied both at the wellhead and at the slick surface to break oil into smaller droplets to expedite microbial degradation. Though the effects of crude oil on survival and cardiac development of early life stages of marine fishes has been established, few studies have looked at the impact of developmental exposure on ecologically relevant behavioral endpoints that relate to both fish feeding and reproduction. Male aggression in the defense of breeding territory, and feeding behavior, play critical roles in both the growth and reproductive success of marine fishes. In our study, sheepshead minnow (Cyprinodon veriegatus veriegatus) embryos were exposed from 1-10 dpf to water accommodated fractions (WAFs) of crude oil, weathered crude oil, crude oil and dispersants, weathered oil and dispersants as well as dispersants alone. Prey capture efficiency was quantified at 60 dpf using brine shrimp nauplii. At 8-11 months the male fish were assessed for aggression-based behavior, using strikes against a mirror. Exposure to the various WAFs had no direct impact on the latency to prey capture, but caused a shift in the bold and shy behavioral phenotypes normally found. Ecologically relevant endpoints such as feeding behavior and male-type aggression behavior on marine model species, such as the sheepshead minnow, will allow for more accurate predictions of the impact of crude oil release on fish populations.

RP180 The Influence of Mixing Energy on the Concentration and Composition of Oil in Laboratory Toxicity Tests

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Water accommodated fractions (WAFs) are laboratory preparations used to simulate oil exposure in aquatic toxicity testing. Standard protocols for preparing WAFs use low- or medium-energy mixing. More recently, a high-energy mixing technique (HEWAF) was developed and used to evaluate the potential toxicity of oil spills, such as the Deep Water Horizon release. Concentrations of total polycyclic aromatic hydrocarbons (TPAH) in unfiltered HEWAFs were one to two orders of magnitude higher than WAFs prepared using established protocols. In addition, HEWAFs had larger percentages of potentially toxic high molecular weight PAHs and alkylated PAHs in comparison to other WAFs. This compositional shift was conserved over a wide range of dilutions. Only a small percentage of the surface water samples collected in the Gulf of Mexico during the active spill period in 2010 were similar in concentration and composition to laboratory HEWAFs. The implications of these compositional differences on potential toxicity will be discussed.

RP181 Comparing the toxicity of crude oil in fresh and saltwater systems: linking chemistry and gene expression

D. Lyons, Univ of Alberta; D. Philibert, K. Tierney, Univ of Alberta / Biological Sciences

Crude oil is highly toxic to fish during early life stages. As an exceptionally complex mixture, oil induces numerous abnormalities in developing fish including cardiac and morphological deformities. This study compares the molecular responses of saltwater and freshwater fish species (sheepshead minnow and zebrafish) to crude oil exposure. We exposed sheepshead minnow and zebrafish embryos to water accommodated fractions (WAFs) of crude oil, weathered crude oil, crude oil plus dispersant, and dispersant alone. We measured gene expression levels in both sheepshead minnow and zebrafish embryos after exposure, targeting genes involved in neurological and cardiac development as well as biotransformation. In our exposures, source oil WAFs had a higher concentration of polycyclic aromatic hydrocarbons (PAHs) than weathered oil WAFs. The combination of dispersant and oil vastly increased the amount of PAHs in the WAFs when compared to oil alone. This increase in PAHs occurred to a greater extent in saltwater than freshwater, indicating that oil and dispersant interact differently in these two aquatic environments. Due to the increase in PAHs, the oil plus dispersant WAF was more toxic to developing fish embryos than the other exposures. Though biotransformation gene expression levels were upregulated in both species in response to oil exposure, they were induced to different extents with zebrafish having a much higher expression level. The results of this study enable us to compare and contrast the consequences of oil spills in both freshwater and saltwater as well as demonstrate the implications of using a chemical dispersant in these environments. We were also able to help establish whether zebrafish, a common model species, are valid models for studying marine oil spills.

Mixtures: Exposure and Toxicity from Combinations of Stressors

RP182 Incoporating bioavailability-based measurements into whole-sediment toxicity identification: An application in South China

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Sediments in urban waterways of Guangzhou, China were contaminated by a variety of chemicals and showed prevalent toxicity to benthic organisms. A combination of whole-sediment toxicity identification evaluation (TIE) procedure and bioavailability-based extraction approaches was used to identify the causes of sediment toxicity. Four of the six sediment samples collected caused 100% mortality to Chironomus dilutus in 10-d bioassays and the potential toxicants were assessed using TIE testing in these sediments after dilution. The results of phase I characterization showed that organic contaminants were the principal contributors to the mortality of the midges in two sediments, and metals and organics jointly caused the mortality in the other two sediments. Ammonia played no role in the mortality for all the samples. Conventional toxic unit analysis in phase II testing identified Cr, Cu, Ni, Pb and Zn as the toxic metals while cypermethrin, lambda-cyhalothrin, deltamethrin and fipronils being the toxic organics. To improve the accuracy of identifying the toxicants, four-step sequential extraction and Tenax extraction were conducted to analyze the bioavailability of the metals and organics, respectively. Bioavailable toxic unit analysis narrowed the list of toxic contributors and the principal toxicants included three metals (Zn, Ni and Pb) and three pesticides (cypermethrin, lambda-cyhalothrin and fipronils). Metals contributed to the mortality in all sediments, but sediment dilution reduced the toxicity and caused metals' contribution to the mortality in two sediments being overlooked in phase I characterization. Incorporating bioavailability-based measurements into whole-sediment TIE testing improved the accuracy of identifying the causative toxicants in urban waterways where multiple stressors occurred and contributed to sediment toxicity jointly.

RP183 Toxicity of manganese in the presence of cadmium or lead to the nematode Caenorhabditis elegans

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Manganese (Mn) contamination from mining industry poses a significant public health concern as Mn is a neurotoxicant and can induce a Parkinson-like syndrome called “manganism”. Existing literature on Mn neurotoxicity as well as regulatory guidance on human exposure mainly focus on single element, without considering the co-occurring metals. Mn contamination sites are usually accompanied with other toxic metals such as Pb and Cd; however, toxicity of Mn in combination with these metals are not well-understood. The current study examined the toxicity of Mn in the presence of Cd or Pb, the two most commonly found companying metals in Mn contamination sites, using a model organism the nematode C. elegans. The central hypothesis is that the presence of Pb or Cd will induce additive or synergistic effects in the toxicity of Mn in C. elegans. C. elegans were exposed to Mn with or without the presence of Cd or Pb, and a series of toxicity endpoints were evaluated, including lethality, reproduction, behavior, lifespan, neurotoxicity, and oxidative stress. Neurotoxicity was evaluated using a dat-1::GFP transgenic strain of C. elegans that expresses GFP in dopaminergic neurons of the worm, which enables in vivo imaging of the neurons for structural and morphological alterations. Overall oxidative stress was assessed using a daf-16::GFP transgenic strain of the worm. Exposure to Mn alone from low µM to low mM levels caused significant toxicity in reproduction, locomotion behavior, lifespan, neurotoxicity and oxidative stress in the worm. The neurotoxicity effects included neuronal body shrinkage, breakage in neuronal processes, and errors in axonal pathfinding in dopaminergic neurons. The oxidative stress was indicated by translocation of GFP from cell cytoplasm to nucleus in the worm. Adding Pb or Cd to Mn induced significant synergistic toxicity effects in C. elegans as shown in most of the endpoints examined. For example, LC10 Mn together with LC10 Cd induced a 65% mortality and LC10 Mn together with LC10 Pb caused a 90% mortality in the worm. Similar synergistic effects were also seen in lifespan, reproduction, neurotoxicity and oxidative stress. These findings suggest that accompanying metals may significantly increase the toxicity of Mn, and hazard and risk evaluation of Mn must take into consideration of the potential synergistic effects with other co-occurring metals.

RP184 Multivariable analysis of ecotoxicity and basic water quality parameters in two watershed areas in Japan: effects of concentration process

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We have attempted to find a trend in various ecotoxicity and basic water quality items in two river watersheds in Japan, one in Yoshino River, Tokushima, and the other in Yodo River, Kyoto-Osaka of Japan. Over three years, we conducted short-term chronic toxicity tests for whole water samples using fish (Danio rerio), daphnia (Ceriodaphnia dubia), and alga (Pseudokirchneriella subcapitata), and Microtox test was also conducted. Basic water quality items such as BOD, COD, NH_4^+-N, and hardness were determined as well as on site measurements of DO, pH, and electric conductivity (EC). This year, we also used two solid-phase cartridges, one is Oasis HLB to concentrate organic contaminants and the other is Nobias Chelate column to concentrate cationic metals to more frequently detect the ecotoxicity, which we failed to detect in most of the samples. Several multi-variable analyses such as principal component analysis (PCA), factor analysis, clustering analysis, and regression analysis were used to find out a trend in spatial variation and in the selected parameters. As with those results we reported last year, we found from the PCA results that BOD, COD, hardness, NH_4^+-N, and EC are in the similar area with relatively high primary principle component while DO is in negative side. Similar trend was found for the factor analysis and the organic compounds originated from human activities such as domestic wastewater are probably the major factors. In contrast, the secondary principle component was higher only for daphnia reproduction/survival, which suggests the toxicity for C. dubia is the very specific item with little correlation with the other basic water quality items and toxicity. The clustering analysis roughly showed the trend with separation of upstream and downstream sites. The regression analysis did not find any significant correlations between water quality items and the C. dubia and algal toxicity. The frequency of detecting toxicity was jumped up by the concentration process but it needs special cautions for concentrating cation metals due to the use of acid and neutralization, which results in the increase of salt concentration to interfere the C. dubia reproduction.

RP185 Real Metals for the real world: A Comparison of Dosing Methods to Determine Soil Quality Guidelines

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Standard soil dosing methods for metal toxicity research poorly represent real world contamination. While laboratory soil toxicity assays involve dosing soils with aqueous metal salts, field contamination is almost exclusively caused by deposition of smelter emissions as complex metal mixtures. Fixed-ratio ray dosing regimens can be used to test the toxic effects of these complex mixtures, whether they are based on regulatory limits, relative toxicities, or environmental concentrations. The metals are mixed in their relative concentrations and toxicity is tested against an organism. Currently, metal salts are added to test soils in a fixed-ratio ray and then the soils are leached with artificial rainwater to remove excess salinity. Unfortunately, metal that has been added to the soil is also leached. While this is not so much an issue for single metal tests, mixtures lose individual metals in disproportionate amounts. The fixed-ratio ray is no longer fixed and therefore flawed, making metal toxicity guidelines based on this approach inappropriate. To remedy this issue we evaluated three methods of creating metal mixtures of lead, copper, cobalt, nickel, and zinc in four soils. The mixtures were applied in three ways: (i) pipetting in aqueous nitrate salts and leaching, (ii) adding commercially purchased metal oxides as dry powders, and

(iii) adding synthetic minerals produced via annealing. The synthetic minerals are created in three steps. First, aqueous nitrate metal salts are mixed in the desired mixture ratio. Ferric nitrate solution is then added to the mixture in a 2:1 molar ratio of iron to the sum of the other metals. A small sample of the mixture is removed and titrated to pH 7.5 using ammonium hydroxide and centrifuged. The remaining metal concentrations in the supernatant are used to adjust the main mixture, and it is then also brought to pH 7.5 and centrifuged. The precipitate is heated to 600?C to remove excess nitrates, and then tested for metal concentrations and added to soil. Metal toxicity will be tested using F. candida, O. nitens, and E. crypticus to see how the different dose methods affect toxicity, possibly through different metal complexes being formed. Solid phases of the metals in each sample will be examined using XRD and compared to field contaminated soil. This study will demonstrate the strengths and weakness of these three different methods of creating metal mixtures in soils for fixed ray analysis.

RP186 Putative mechanisms of atrazine's toxic interactions with other chemicals

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Atrazine is an herbicide with several known toxic effects, including interactions with other chemicals. For example, atrazine increases the toxicity of several organophosphates in some invertebrates and conversely reduces the toxicity of triclosan and DHA to D. magna in a concentration dependent manner. Our preliminary research suggests atrazine provides protection from triclosan through induction of glutathione S-transferases (GSTs), potentially due to activation of the xenobiotic-sensing nuclear receptor, HR96. We performed RNA-seq to determine if atrazine is inducing protective effects by inducing several detoxification enzymes and to estimate other interactions. Our RNA-seq analysis demonstrates induction of GSTs and several other detoxification enzymes including CYP370A9, glucosyltransferases, and alcohol dehydrogenase. Pathway analysis is underway and we have confirmed induction of GSTS1, GST1-1, CYP370A9, and ALDH by qPCR. Based on our RNA-seq data, we can hypothesize as to which environmentally relevant toxicants may have interactions with atrazine depending on whether they are activated by CYPs or detoxified by CYPs and other detoxification enzymes. We recently performed acute toxicity tests to determine individual LC_{50} and hillslope values. We are currently performing toxicity tests with binary mixtures containing atrazine. Therefore, we can compare observed toxicity with expected toxicity using the Computational Approach to the Toxicity Assessment of Mixtures (CATAM) independent joint action model and assess whether atrazine is exerting antagonism, additivity, or synergistic toxicity in accordance with our hypothesis.

RP187 Developmental and interactive effects of arsenic and chromium to developing Ambystoma maculatum embryos: toxicity, teratogenicity, and uptake

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Anthropogenic activity has contributed to elevated concentrations of arsenic 12 and chromium in the environment. Both arsenic and chromium are known to induce toxic effects in exposed organisms, including amphibians. The spotted salamander, Ambystoma maculatum, may be useful for showing developmental effects caused by exposure to these metals as larvae develop in water that may contain arsenic or chromium. After exposure to environmentally relevant arsenic and chromium concentrations using Na2HAsO4 * 7H2O and Na2Cr2O7 * 2H2O, larvae showed a different pattern of whole-body arsenic and chromium from individual and mixture19 exposure. 12 day LC50 concentrations from individual As(V) and Cr(VI) exposure in Ambystoma maculatum larvae were 261.17 mg/L and 71.93 mg/L, respectively, while 12 day EC50 concentrations were 158.82 and 26.05 mg/L, giving teratogenic indices of 1.64 and 2.76. A mixture of arsenic and chromium yielded a teratogenic index of 2.78, indicating that these metals are also teratogenic when exposed as a mixture. These results show that Ambystoma maculatum may be a useful indicator of environmental toxicity from arsenic or chromium exposure.

RP188 Time to sampling after capture impacts site-specific ovarian steroid production in rainbow darter (Etheostoma caeruleum)

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Studies on male fish in the Grand River (Southern Ontario, Canada) have shown a variety of reproductive issues such as increased intersex condition (ova-testes) and vitellogenin production, and decreased gonadsomatic index and testosterone production. Many of these effects have potentially been linked to proximity to the effluent outflows of wastewater treatment plants (WWTP). WWTP effluents are complex mixtures that contain numerous chemicals with the potential to disrupt the endocrine systems of exposed fish. This study investigated the sex steroid production levels of female rainbow darter (RBD, Etheostoma caeruleum) at two locations in the Grand River: a location upstream (reference) of the Waterloo WWTP effluent outflow and a site that is downstream of the outflow (exposed). Secondly, this study examined the impact of the collection method on the steroid production. Given that field collections of small-bodied fish in rivers often employ electrofishing methods and that stress is known to affect steroid levels, collected fish from both locations were sacrificed and ovaries removed at 1, 7, and 24h after electroshocking. Ovarian tissue was cultured in vitro for 24h with or without the addition of human chorionic gonadotropin (hCG), an analog to endogenous luteinizing hormone that stimulates steroid production. Reference fish sacrificed 1h post-collection had stimulated ovarian estradiol production levels of 54.4 ± 4.8 pg/mg of tissue, which was significantly higher than exposed fish sampled after 1hr (34.3 ± 2.2 pg/mg). After 7h post-collection, basal estradiol production levels for reference fish ovarian tissue (5.0 ± 0.44 pg/mg) were significantly higher than those of exposed fish (3.1 ± 0.40 pg/mg). Over the 24h, stimulated estradiol levels decreased significantly in reference fish ovaries (1h 54.4 ± 4.8, 7h 40.7 ± 4.0, and 24h 25.6 ± 2.9 pg/mg). After 24h, there were no significant differences in basal or stimulated estradiol production levels between the reference and exposed fish ovaries. These results suggest that standardized collection and sampling methods may be important to interpreting reproductive steroid levels of fish collected through electrofishing and in the identification of site-specific differences. The impact of time held post-shock may be a stress-related response with enough magnitude to impact the ability to detect reproductive endocrine impacts of point/non-point sources on small-bodied fish and is being further investigated.

RP189 Evaluation of Chemical Mixture Toxicity Predictability with High Dimensional Toxicogenomic Data Using Parallel Factor (PARAFAC) Analysis

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Predictive toxicity assessment of chemical mixture is challenging and complex due to unknown interactive modes of action (MoA) including concentration addition, independent action, synergistic, or antagonistic models. Toxicogenomic technology combined with bioinformatics have the potentials to transcend the limitations of conventional toxicological approaches for predicting mixture toxicity. However, toxicogenomic based mixture toxicity prediction has hardly been explored due to limitations including lack of quantitative dose-dependent toxicogenomic data, limited established theoretical and modeling framework, data complexities due to large no. of variables associated with small sample size, and lack of consensus in computational methodologies. Here we apply tensor decomposition method- Parallel Factor (PARAFAC) to toxicogenomic data (temporal transcriptomic data of 120 biomarkers of all known stress pathways, over 2-hr exposure time at 5-min interval, generated by GFP-fused whole-cell array of E.coli K12, MG1655) for predicting molecular toxicity of a binary mixture of two metals- As and Cr. PARAFAC model decomposes 3-D toxicogenomic data (biomarker x time x chemical dose) of the mixture and individual chemicals separately into multiple components/factors. Decomposed PARAFAC factors represent chemical-specific distinct fundamental biological and molecular features, which are further characterized via gene set enrichment analysis to reveal significantly impacted pathways (e.g., DNA damage, protein damage, and so on) and therefore implied dominant toxicity mechanisms represented by each factor. We have further demonstrated the efficacy of biomarker factor loads of individual chemical models as prediction parameters to predict mixture toxicity. Significant prediction models with higher confidence ($p< 0.05$) have been found to estimate quantitative mixture toxicity endpoint from biomarker factor loads of individual chemical models using linear regression. However, estimation models at the pathway level using pathway loads, achieved by aggregating biomarker loads, as prediction parameters are not significant ($p>0.05$). This study explored a novel application of PARAFAC analysis in predicting mixture toxicity at molecular level that can provide significant insight into toxicogenomic based mixture toxicity. Further study is on-going to include more mixtures with different MoA, and varying biomarkers to contribute to the knowledge gap in predictive mixture toxicity.

RP190 Too thick to drink, too thin to plow: Chemical mixtures in the Elkhorn River, Nebraska

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Agrichemical loading to surface waters in the Midwestern United States is driven by surface runoff. From April until July, agrichemical concentrations in surface waterways, such as the Elkhorn River, peak following rainstorm events. During dry years, the rain events are discrete, and episodes of chemical exposure are separated by periods during which the chemical exposure returns to baseline conditions. During wet years, the series of episodic exposures fuse into a continuous exposure of lower concentration. In both cases, runoff does not contain one agrichemical, but rather a suite of co-occurring herbicides. Furthermore, the overall concentration of the suite of herbicides is influenced by abiotic factors including water temperature, photoperiod and the overall load of suspended sediments. The primary biological impacts of the herbicide mixture are reproductive, as fish exposed to the raw water and sediment experience reductions in the expression of estrogen responsive genes. Biological impacts are not uniform across the entire spring season, but rather are influenced by rainstorm runoff and river discharge. Our research on the Elkhorn River suggests that regulation of herbicide concentration in surface waters in Nebraska needs to take episodic events into consideration along with overall average chemical concentration. In keeping with the session's theme, the presentation will address the integration between the chemical mixture found in the Elkhorn River and the environmental conditions (temperature, discharge, suspended solids) that influence the behavior of those chemicals.

RP191 Does competition for food influence the toxicity of heavy metal mixtures?

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The toxic effects of metals on individual species of soil invertebrates are well documented in the literature. However, impacted soils typically contain a mixture of metals and impact a community of invertebrates. Here, we evaluated the effects of individual metals and metal mixtures of Cu, Co, Ni, Zn and Pb in a fixed-ratio ray dosing regimen on the reproduction of Folsomia candida and Oppia nitens. Mites and collembolans were tested for their response to metals separately as well as with both organisms present in the test vessel. Single metals were dosed with 11 different concentrations determined based on F. candida EC50 for each metal and mixture dosing were performed with 10 different ratios simulating the ratios observed in contaminated sites and guideline values each with 8 different doses. The selected dosing regimen will allow the calculation of dose and ratio dependant deviations from concentration addition and the single vs. combined exposure will allow observation of any indirect effects due to competition. This experiment is the first study on the role of competition in soil invertebrate reproduction and survival when exposed to both single and mixtures of metals. The total metal exposure experiments should be finalized in the July 2016.

Linking Science and Social Issues – Poster Only

RP192 Bridging the Gap Between Cannabinoid Toxicity and Public Perception

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Cannabis has been used for several millennia as a medicinal therapeutic. Until the enactment of the 1937 Marijuana Tax Act, cannabis was being used widely across the United States and found in numerous pharmaceuticals. Currently, medical cannabis has shown potential in many areas of treatment (i.e. insomnia, loss of appetite, cancer, Dravet syndrome, etc), however, it is a Schedule I drug in the United States and illegal under Federal Law. While public perception of medical cannabis varies, empirical toxicological data is lacking and widely conflicting. Therefore, the consequence of developmental exposure to cannabinoids on the potential etiology of subsequent adult or multigenerational toxicity is currently unknown. Herein, we examined the potential toxicities of two cannabinoid constituents currently being used for therapeutic purposes, Δ9-tetrahydrocannabinol (THC) and cannabidiol (CBD), on zebrafish morphology, behavior, and morphogenesis/neurogenesis. Our results indicate phenotypic dysmorphologies, enhanced locomotion, and differentially expressed genes imperative for normal development. THC and CBD shared similar adverse morphologic and behavioral outcomes, but lacked similarity in terms of differential gene expression in key morphogenesis/neurogenesis genes. For example, c-fos was significantly upregulated in THC and CBD exposed zebrafish, while dazl was significantly upregulated following only THC exposure. Additionally, CBD was greater than 6 times more potent than THC, LC_{50} 0.53 ± 0.19 mg/L and 3.65 ± 0.67 mg/L, respectively. Through this work we are gaining additional insight into potential mediating mechanisms of cannabinoid toxicity which can be used to influencing public perception via empirical evidence. Supported by NIGMS, Grant Number P20GM104932.

RP193 Evolving Products of Consumer Convenience and their Environmental Implications

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Products of consumer convenience have and are continuing to change the consumer landscape. These products have a common thread of saving time and effort while producing a similar end result or experience when compared to their conventional counterparts. Modern day examples in various stages of technological development and consumer adoption include disposable diapers, single serve coffee pods, and compostable silverware. At the same time, these products have the potential to exhibit significantly different environmental, economic, and social impacts when compared to their conventional counterparts. A midpoint life cycle assessment was completed to evaluate the environmental impacts of consumer convenience on coffee. Three generalized coffee brewing systems were evaluated: drip filter, french press, and single serve coffee pod (a product of consumer convenience). Inventory data with respect to the raw materials and use phase were collected through tear-down and experimentation with the three brewing systems. Potential for material change and alternate end of life scenarios for the coffee pods is also discussed. In evaluating the environmental impact of growing and processing the coffee in Brazil (one of the major coffee producers) the most significant contributors to the life cycle impact were found to be fertilizer, pesticide, and diesel utilized in the growing and harvesting of the coffee plants. As expected, for the conventional coffee brewing methods (drip filter and french press) the most significant environmental impacts across all of the impact categories considered were due to the coffee grounds themselves and the electricity utilized to brew the coffee. It was expected that for the single serve pod style coffee that that additional materials required to produce the pod (foil and plastic cup) would dominate the life cycle impact, however, that was found not to be true. In fact, brewing coffee with a single serve pod was found to have a lower environmental impact in all of the impact categories considered than the conventional brewing methods. This is largely due to the coffee pod containing less ground coffee to produce the volume of coffee drink, and using less energy during the brewing process. This is a valuable case study in that it suggests that products of convenience do not inherently have a larger environmental footprint than their conventional counterparts.

RP194 Impact of Environmental Contaminants on Childhood Education; Synthesis of Ecological Systems theory, Piaget and Vygotsky Education theories

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The impact of environmental contaminants on childhood education is becoming very obvious in the developing countries like Nigeria. Mining may cause environmental issues ranging from waste rock and tailing disposal, land disturbance, dust and noise, to environmental pollution. If not managed well, any of these could adversely affect the health and educational development of the children living near mining operations. Miners and their families are exposed to harsh working conditions for minimal income in a high risk context, endangering their health as well as often the surrounding environment. These contaminants, such as lead, have been reported to create very serious impediment in childhood education, particularly their cognitive experiences. In this work, we evaluated lead concentrations in soil and plants in a lead mining community of Ebonyi State, Nigeria. We also reviewed the literature on the effect of environmental lead in other to link environmental contaminants with the mechanisms of motor development, which promotes participation and active learning in children, in accordance with Piaget's theory of cognitive development, an extremely important domain in early childhood education. In Vygotsky's wiew, childhood development was a process and could be a period of crisis in cognitive development in an event that can create a perturbation on the child. For example a qualitative transformation in the child's mental functioning could be underpinned by toxic substances in the environment. All these are wrapped up in The Ecology of Human Development. Through this work, an approach or forum for a coordinated assessment of environmental contaminants as it affects childhood education would be developed for the purpose of solving the problems and needs of the environmental pollution-endangered communities, since it has been posited that environmental contaminants, exemplified by lead, have a persistent and irreversible effect on intelligent quotient (IQ) even during the adult years.

RP195 Land Use-Sustainability Browser: A Decision Support Tool Linking Enviromental Science to Social Outcomes

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With the increasing scale, scope, and complexity of problems addressed by environmental toxicologists and chemists, the profession has welcomed and benefited from the input and perspectives of social scientists. The influence of the social sciences is evidenced, in part, by the development of decision support tools (e.g., EnviroAtlas[1] and the EcoHealth Relationship Browser[2]) and indices (e.g., Environmental Quality Index, Human Well-Being Index) to quantify and substantiate the interconnections between environmental, social and health indicators. These tools are designed to better communicate these interconnections and many allow comparisons between locations over a range of metrics. A number of decision support tools developed by EPA provide ready access to information, references, and facilitate visualization of data to inform community choices regarding sustainability and the resilience of urban and rural environments. We report the development, status and functionality of a new relational browser on land use and sustainable development. The Land Use-Sustainability Browser is supported by a data base of more than 1,200 peer-reviewed publications on the importance of land use as both a positive and negative driver of sustainable development. Linkages between land use and transportation, materials and waste management, buildings and infrastructure are included as major influences on sustainable development. More than 200 references are cited as evidence to support summaries of the literature related to land use and sustainability. We demonstrate the functional capability of the browser for scenarios relevant to environmental toxicology, chemistry and social outcomes. [1]www.epa.gov/enviroatlas [2]https://www.epa.gov/enviroatlas/enviroatlas-eco-health-relationship-browser

RP196 Modern history of hypoxia in Narragansett Bay: the geochemical record

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Increased inputs of nitrogen and other nutrients to estuarine and marine ecosystems from agricultural runoff, urbanization and suburbanization have resulted in degradation of water quality, including increased frequency and severity of hypoxia. While much work has been conducted in recent years to characterize the spatial and temporal extent of hypoxia in coastal systems, the historical record of hypoxia in such systems is much less well known. The current work examines the history of hypoxia in upper Narragansett Bay and nearby Greenwich Bay, an urbanized estuary in the northeastern U.S., through vertical profiles of geochemical markers in sediment cores. Concentrations of molybdenum (Mo), a quantitative surrogate for direct measurement of hypoxic conditions in overlying waters, reveal periods of more frequent hypoxia that vary spatially and temporally throughout the Bay. Variations in the degree and extent of hypoxia are related to changes in the population and anthropogenic inputs to the Bay from the surrounding watersheds, as well as estuarine circulation patterns within the Bay. The more urbanized upper bay shows greater frequency of hypoxia at the turn of the 20th century with conditions improving through mid-century and recurrent hypoxia thereafter. Hypoxia was less prevalent in the early 20th century and increased substantially from ~1950 to the present in Greenwich Bay, which has a more suburban watershed. Carbon and nitrogen concentrations and isotope values reflect increased nitrogen enrichment and productivity in the Bay in the 2nd half of the 20th century. These results can help inform study of the environmental responses to societal activities that may degrade or improve water quality.

RP197 Risk Assessment on four Artificial Sweeteners, alternatives to reduce excessive Sugar consumption

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Food industry is gradually developed, and the use of food additives that are necessary to improve the food preservation and distribution has been incresed too. Standards and specification of Food additives have been established and managed by the Ministry of Food and Drug Safety(MFDS) in Korea. The interest in safety of food additives also has been increased because of the potential health hazardous risk from the use of synthetic addivies. There was an issue that excessive sugar intake might cause adverse effets on blood glucose by generating diabetics, metabolic syndrome and obesity. As the result, the interest in sweeteners has been raised, but some reports that artificial sweeteners may cause adverse effects including fetus obesity are increasing the anxiety on health effects otherwise. Even though some sweeteners were already permitted to use in food, and certified its safety by the MFDS in Korea, Joint FAO/WHO Expert Committee on Food Additives(JECFA) and European Food Safety Authority(EFSA), safety reassessment on four sweeteners (Sodium saccharin, Acesulfame potassium, Aspartame, Sucralose) was conducted to relieve the public from safety anxiety. The hazard identification of the sweeteners was come up with summarizing the safety information from JECFA and EFSA New information of the sweeteners was gathered by research papers. Based on the large number of toxicological studies requesting the safety evaluation of the sweeteners, the No Observed Adverse Effect Level (NOAEL) is identified for the most sensitive effect in the most proper animal species. A safety factor of 100 is normally applied to the NOAEL in order to establish the ADI for humans. The level of safety on these sweeteners was evaluated by comparing among the ADI data established by the JECFA or EFSA. For the exposure assessment of the sweeteners, we used poundage method based on Korea National Health & Nutrition Examination Survey and guidelines on safe use of sweeteners. In this study, four sweeteners, Sodium saccharin, Acesulfame potassium, Aspartame, Sucralose, reassessed were all evaluated as not hazardous to human.

RP198 The importance of medication disposal for consumers. (But they don't know or seek information about proper methods)

J.S. Punzi, CHPA / Regulatory and Scientific Affairs

Over-the-counter (OTC) products comprise the majority volume of pharmaceuticals and personal care products purchased by consumers. Proper disposal OTCs is important for safety and environmental concerns. To identify consumer awareness, knowledge and behavior of disposal practices, the CHPA and its Educational Foundation engaged Nielsen Research/Harris Poll for a national survey During February 3-9, 2015, of adults age 18 years and older who used/purchased OTC medicines in the past 6 months. The online survey instrument included 49 questions, 9 regarding disposal. Data were weighted for propensity to be online and U.S. proportions for all demographic variables. From 2002 respondents (54% female), 95% had used/purchased medical pills/tablets/capsules, 92% personal hygiene drugs,73% vitamins/supplements, 50% medical liquids, 48% topical medical creams/ointments. We determined that 50% of the respondents "typically" dispose of expired/unwanted in the household trash and 20% flush them down the sink or toilet. Only 7% return them to local take-back programs or pharmacies (6%). While 90% of the respondents agreed "the way someone disposes" is important, and 49% "wish they knew more" about "proper" disposal, 62% have "never sought" information. Even though half of consumers currently dispose of OTCs in household trash which usually terminates in EPA- regulated landfills, too many continue to dispose of unused/unwanted OTC's by flushing, and even fewer utilize existing "take-back" programs. There is clearly a need for better public education about proper disposal of pharmaceuticals and personal care products.

RP199 Tox on Tap: Changing Public Perceptions of Science

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Public perception of science is heavily influenced by the way in which research and scientific development is communicated. Though always present to some degree, in the previous few years the communication gap between scientific researchers and the general public has widened significantly. This has contributed to a general negative shift in the public perception of science, especially in emerging and controversial areas of scientific development. Tox on Tap (ToT) aims to combat this communication gap, by bringing scientists from academia, government, and industry together with members of the local community to discuss scientific developments and recent issues in hot-topic toxicological and environmental areas. A non-profit, volunteer-run initiative, ToT aims to make science interesting and understandable to the general public by using a storytelling/discussion-based approach mirroring that of Café Scientifique. Primarily run during the academic year at a local pub, these events have been met with great public interest. Attendance has ranged from 40-120 individuals per event, with topics discussed having included mercury exposure via seafood, rhino poaching, prion diseases, and environmental teratogens. The informal and friendly atmosphere of these discussions has promoted audience participation and education about current scientific research. We would like to share our successful stories of effectively communicating science, inspiring the public through our events, and increasing perception of the scientific community.

RP200 Environmental Implications of Efficiency: Fuel Efficient Vehicles and the Rebound Effect

J. Mahoney, Univ of Wisconsin – Madison / Civil Environmental Engineering; A. Hicks, Univ of Wisconsin – Madison / Civil and Environmental Engineering

One of the pressing problems facing humanity in the 21st century is a changing climate, with the transportation sector emitting approximately a quarter of all emissions. Efforts to reduce emissions from transportation fall into two camps: market-based approaches of increasing fuel prices and improving fuel efficiency standards through legislation. Producing vehicles with improved efficiency allows consumers to travel a given distance using less fuel, leading to decreased emissions. An important consideration while quantifying the fuel savings from more efficient vehicles is to determine the rebound effect. The rebound effect occurs when an increase in efficiency of a good leads to increased consumption of that good. Accurately estimating the rebound effect in vehicles will show what percentage of the theoretical fuel savings is realized. This determination will offer insight into whether regulations pertaining to efficiency will lead to overall fuel savings. To gain understanding into the fuel efficiency rebound effect, consumer surveys were administered to gather data on driving habits and demographics. This study utilized the data to estimate the transportation rebound effect for various groups of participants. Groups were established based on demographic data, such as comparing rural and urban households and various income levels. The variations in the magnitudes of the rebound effect among the groups were analyzed to investigate factors that influence driving behavior and to what extent they affect fuel price elasticity. It is anticipated that comparing the rebound for households on different ends of demographic categories will reveal significant differences in their respective rebound effects. Of particular interest was comparing rural and urban households and investigating the rebound differences. An important factor in this comparison is the availability of alternate modes of transportation, or lack thereof; when fuel prices increase, urban households can readily adopt alternate transportation or revert from it when fuel prices fall, whereas rural households may not have that option. These findings can be used to identify how different groups of drivers respond to improvements in fuel efficiency. Better understanding this behavior will provide discernment into the efficacy of using fuel efficiency mandates to reduce transportation emissions.

Aquatic Toxicology and Ecology – Poster Only – Part 4

RP201 Water supply resilience through watershed management under climate change regime: An excerpt from the Brahmaputra River watershed of India

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In the present era of high population growth, rapid urbanization and climate change, uncertainty of natural phenomenon and land use pattern are rapidly increasing and posing serious threat on drinking water supply from both quantity and quality perspectives especially in a country like

India. The aim of this study was to achieve sustainability in the water resource management by understanding the governing processes of water storage and supply on watershed scale followed by developing integrated efficient operating system. The study has been carried out in the Brahmaputra Basin by assessing the current urban water use in the river watersheds through questionnaire survey, review of available scientific and government organization's reports. Further, health risk from the current water sources was estimated through measuring concentrations of fecal coliform and E. coli so that a new strategies of sustainable water supply under climate change scenario may be evolved. Results suggest that the most crucial step for managing the water resource would be an extensive and in-depth study of the impact of climate change on water resources on watershed scale. Urbanization results in severe environmental deterioration because countermeasures, which need to wait for policy-makers' decision, cannot catch up with the change.Much advancement has been made in this field but the difficulty lies in the application of the existing information to arrive at a solution for this. Decision-making needs to consider several aspects of health, environment, economy, socio-culture and technical function within a framework that includes interactions between users, organizations and technology. Applicability of results seems universal as the climate change evidently affects the global precipitation pattern which can lead to societal conflicts. Hence the need of the hour is to adopt mitigatory approaches which can at least diminish the effect of climate change to a certain extent.

RP202 Is harmonization of the number of test concentrations possible in the OPPTS and OECD fish bioconcentration tests?

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In 2012 the OECD fish bioconcentration test guideline (TG 305) was revised to incorporate a dietary bioaccumulation test and to utilize fewer fish by providing the option of decreasing the number of test concentrations, from two to one, where appropriate. Until this revision the USEPA OPPTS 850.1730 and OECD TG 305 test guidelines were fully harmonized. The OPPTS test guideline was written in 1996 and, to this date, remains a draft test guideline. An analysis by Crop Life America (CLA) of the results of fish bioconcentration studies with crop protection products compared the bioconcentration factors (BCFs) derived from two test concentrations. This augments a similar effort conducted in Europe which resulted in the revision of OE CD TG 305 to include an option for reducing the number of test concentrations required. The hypothesis is that it is sufficient to conduct the fish bioconcentration test with one test concentration based on supporting data from studies previously conducted with two test concentrations. The use of one test concentration provides acceptable and relevant fish bioconcentration results and reduces the number of vertebrate organisms tested.

RP203 An evidence base to support the one concentration approach in fish bioconcentration studies for plant protection products and general chemicals

N. Burden, NC3Rs; S.K. Maynard, Syngenta / Environmental Safety; L. Weltje, BASF SE / Crop Protection Ecotoxicology; J. Wheeler, Dow Agrosciences

Fish bioconcentration studies are conducted to address various regulatory requirements. These tests result in the generation of a bioconcentration factor (BCF), which is used to determine whether substances are bioaccumulative and/or need further consideration regarding accumulation in the food chain and for secondary poisoning assessments. Bioconcentration tests are resource and animal intensive, often requiring more than 100 fish per study. Replacing, reducing or refining the use of fish for BCF testing would therefore have multiple benefits, including improved efficiency, reduced costs and supporting animal welfare considerations. Since its revision in 2012, the OECD Test Guideline 305 'Bioaccumulation in fish' includes the option to use one exposure concentration rather than two, providing there is scientific justification. However, two concentrations may still be required for some regulatory purposes. To determine whether the one concentration approach could be justified for plant protection product (PPP) actives and general chemicals, BCF values resulting from low and high exposure concentrations were compared for 55 PPP actives and 236 general chemicals. Within this dataset we also explored the BCF values generated for ionisable substances. Overall, there was no statistically significant difference in the BCF values from low and high exposure concentrations. This suggests that the testing of one exposure concentration is sufficient to reliably estimate the BCF value. The relationship between the low and high exposure concentration BCF values was particularly strong for BCFs $\geq$ 1000 L/kg, which is beneficial as only chemicals with relatively high BCFs (e.g. > 2000 L/kg, for the EU 'B' criterion) may require regulatory action. This analysis provides a data-driven rationale for using the one test concentration approach for PPP actives and general chemicals, and where applied would decrease the numbers of fish used by one third.

RP205 Evaluation of the Daphnia magna Four-Day Survival and Growth Test Method

N. Love, S. Skigen, D. McCausland, GEI Consultants, Inc.

In 2009 James Lazorchak, Mark Smith, and Herman Haring presented a new test method based on the growth endpoint for the Daphnia magna species. The Daphnia magna four-day survival and growth test method was developed to provide an additional option to evaluate the potential for sublethal toxicity in the aquatic environment (Lazorchak, 2009). Current methods for the species involve an acute exposure of only 24 to 96 hours, or long-term chronic exposures of 21 to 28 days. The proposed method would allow a shorter duration test while still incorporating the sublethal endpoint. Recently, many dischargers in the Western United States have been struggling to meet whole effluent toxicity (WET) sublethal limits for C. dubia due exclusively to interference from the total dissolved solids (TDS) components in their effluent. While the four-day D. magna method has not been approved by the USEPA, we have begun to evaluate concurrent chronic C. dubia and four-day D. magna tests in various waters in Colorado to determine whether the method could potentially isolate the TDS component while still providing an estimation of true toxicity. Through the expanded use of the method, we have conducted an extensive evaluation of the method requirements and, in turn, have formulated several key observations and various suggested modifications to the method should it ever be considered as an alternate test method for WET testing. Our evaluation focused primarily on control performance criteria requirements and the data required to demonstrate good performance will be presented.

RP206 Microtox: Bioanalytical Tool in Water Quality assessment. Evaluation of toxicity of samples from Cauca-River-Colombia

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Bacterial Bioluminescense inhibition assays (Microtox) are the most widely used assay for measurement of citotoxicity in water samples. This type of assays utilises the light emission in naturally bioluminescent bacteria (Vibrio Fischeri) as a measure of overall celular energy status and health. A decreased light output indicates interference with energy metabolism and overall celular health, reflecting the combined baseline toxicity of all chemicals in the sample. Bacterial Bioluminescense inhibition assays are therefore very suitable for screening of overall non-specific toxicity. In addition to the low cost and simplicity of these assays, their applications in water quality testing are wide ranging and well represented in the literature providing a large volume of comparative information. The water types tested with the Microtox, for example, span across efluents of coal gassification, oil refineries, pulp mills and sewage treatment plants as well as environmental waters and drinking water. Microtox was used to evalueted the quality of Cauca River from

Colombia. In Colombia, the Cauca River is the second most important artery fluvial. The Valley formed by the Cauca River constitutes the axis of development of different socioeconomic activities in the southwestern region of Colombia, as are power generation, sand extraction, drinking water supply, agricultural, domestic and industrial use. However, these activities impact negatively the ecosystem causing deterioration of the quality of river water. Sediments samples were taken from eight sampling points in across of the Cauca River in the Colombian southwest. Considering the presence of potentially toxic substances, such as pesticides, PAHs and heavy metals, aqueous and organic extracts from Cauca River were used to assess toxicity in sediments by using the photo-luminescence bacteria Vibrio Fisheri as screening response variable. Overall, the Microtox approach appears to be rapid and cost effective for on-site hotspot identification, and may increase understanding of hazards associated with heavy metal and organic contaminants in these waterways.

RP207 Rapid ecotoxicological bioassay using delayed fluorescence in the marine cyanobacteria Cyanobium sp. (NIES-981)

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@font-face font-family: “Cambria Math”; @font-face font-family: “Yu Mincho”; @font-face font-family: “@Yu Mincho”; @font-face font-family: “Osaka”; @font-face font-family: “@Osaka”; p.MsoNormal, li.MsoNormal, div.MsoNormal margin: 0mm 0mm 0.0001pt; text-align: justify; font-size: 12pt; font-family: “Yu Mincho”; .MsoChpDefault font-family: “Yu Mincho”; div.WordSection1 A general method for testing the toxicity of chemicals on algae was described in the 1984 OECD test guidelines (algal growth inhibition test TG201), in which growth inhibition at 72 h after exposure is used to estimate the effective concentration (i.e., EC_{50}) of chemical. Although this test reliably and directly measures algal growth, it is time consuming and expensive because it requires counting the cells every 24 h for up to 72 h of exposure, or more, using an electronic particle counter or under a microscope. The use of delayed fluorescence (DF) intensity as an endpoint for rapid estimation of the effective concentration (EC_x) has been reported as an alternative to standard growth inhibition (at 72 h after exposure) in some fresh water algae including Pseudokirchneriella subcapitata. In marine algae, although an approach of bioassaying using DF measurements has not been performed yet, its development would provide many benefits for marine environmental risk assessment. In this study, we selected marine cyanobacteria Cyanobium sp. (NIES-981) as a test algal species, which is closely related to genera Synechococcus/Prochlorococcus known as major primary producer in marine environment. We knew that this species grows with high efficiency under a culture condition in the preliminary study, and therefore considered that it is candidate for the test species. As a first step, we showed that this species is valid for a standard growth test (72-h growth inhibition test) using five chemicals (3,5-DCP, simazine, diflufenican, $K_2Cr_2O_7$,and $CuSO_4$). Next, we compared EC_{50} values and low-toxic-effect values (EC_{10}, EC_5, and NOEC) of five test chemicals based on the standard growth test with EC_{50} values obtained by a test with a 24-h time period using DF intensity. Finally, we discuss the possibility of rapidly estimating the toxicity of chemicals by DF measurement in Cyanobium sp.. Based on comparisons of the two dose-response curves and the EC_{50} values, we conclude that DF intensity is useful as an endpoint for rapid estimation of EC_{50} in Cyanobium sp..

RP208 Conversion of estrone to estradiol in male fathead minnows: Implications for assessing risk

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Estrogens are frequently observed in aquatic environments associated with anthropogenic influence, such as agricultural runoff and wastewater treatment effluent. While 17β-estradiol (E2) is the most potent naturally-occurring estrogen, estrone (E1) is often found at higher environmental concentrations. However, exogenous sources of E1 could potentially be converted to the more potent E2 through the action of endogenous 17β-hydroxysteroid dehydrogenase activity, specifically, the 17β-hydroxysteroid dehydrogenase type 1 isoform (HSD17B1). Observation of increased plasma E2 concentrations without measureable changes in aromatase (cytochrome P45019a) expression in male fish caged in ambient waters containing elevated concentrations of E1, but low or non-detectable concentrations of E2, suggested this may be occurring in the field. If so, exogenous E1 may have a greater impact on reproductive function in aquatic vertebrates than previously assumed. The present study was conducted to evaluate this hypothesis. Male fathead minnows (Pimephales promelas) exposed to aqueous concentrations of 16.7, 50, and 150 ng E1/L in the laboratory exhibit significantly ($p< 0.05$) elevated plasma E2 concentrations relative to control. Plasma testosterone (T) was elevated at a low E1 exposure concentration (1.8 ng E1/L) and depressed at the highest level of exposure (150 ng E1/L). Additionally, vitellogenin (VTG) mRNA expression was significantly elevated at concentrations of 50 and 100 ng E1/L. Results suggest that when assessing potential risks of environmental estrogen mixtures to fish, it may be necessary to assume that potency of measured E1 is, in fact, equivalent to that of E2. The contents of this abstract neither represent, nor necessarily reflect official USEPA policy.

RP209 Preparation of Water Accommodated Fractions Using a Passive Dosing Technique

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In the assessment of the toxicity of complex mixtures (UVCBs) with limited aqueous solubility, the preparation of Water Accommodated Fractions (WAFs) is the generally advocated method of test solution preparation (OECD 23). The preparation of WAFs is not without its difficulties as great care needs to be taken to ensure that undissolved, dispersed test item is not present in the final WAF used for testing as this can exert physical effects on the test organisms. Chemical stability of the WAF is also an area where questions can arise as the method of preparation does not lend itself to the use of continuous flow-through dosing techniques. The use of a passive dosing technique to prepare WAFs of various petrochemicals was investigated to determine whether this technique would allow for the easy preparation of WAFs where undissolved/dispersed test item would not be present. Chemical analysis of WAFs prepared using both the traditional approach and the passive dosing technique was performed to determine whether similar chemical profiles in the WAFs prepared using the different methods were obtained. Stability of the WAFs was also determined over a suitable time period to be used in standard aquatic toxicology studies.

RP210 Toxicity Identification Evaluation of Textile Dyeing Effluents Treated by Fenton Process

J. Na, Korea Univ; J. Jung, Korea Univ / Environmental Science and Ecological Engineering; G. Nam, Korea Univ / Dept of Environmental Science Ecological Engineering

Textile dyeing wastewater contains many recalcitrant compounds including dyes, surfactants, resins, synthetic sizing compounds, resulting in a threat to human and animal health. This wastewater is commonly treated by biological treatment followed by an advanced oxidation process such as Fenton oxidation. Most of previous studies have been conducted to evaluate the removal of organic compounds and color, without consideration of toxicity of textile dyeing effluents. In this study, ecotoxicity of textile dyeing effluent was identified using Daphnia magna by the TIE (toxicity identification evaluation) procedures. Overall, the analysis showed that Fe and Zn are the key toxicants in textile dyeing effluents, which may be originated from the Fenton process. In addition, ill-defined organic toxicants likely dyes and surfactants also contributed to the observed toxicity of effluents significantly.

RP211 Reducing the number of fish used in acute toxicity testing: Incorporation of the Fish Embryo Acute Toxicity test into the threshold approach

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The assessment of aquatic toxicity is required in various regulatory frameworks and has a huge impact on the number of fish used around the world. In 2011, nearly 180,000 fish were used for toxicological and other safety assessments in Europe. Large numbers of aquatic toxicity tests are required for REACH and, with the 2018 registration deadline fast approaching, strategies to reduce the number of aquatic toxicity tests are urgently needed. The acute fish toxicity test (AFT, OECD Test Guideline [TG] 203) is one of the most frequently used aquatic toxicity tests worldwide. Because death is the endpoint, animal welfare is a significant concern, making the goal of reducing the number of animals used a priority. Applying the threshold approach (OECD Guidance Document 126), where an initial fish test is conducted at one concentration derived from test responses in Daphnia and algae and continued testing is triggered only if mortality is observed at this threshold concentration, can significantly reduce the number of fish used in the AFT. Furthermore, the Fish Embryo Acute Toxicity Test (FET, OECD TG 236) provides a significant refinement to the AFT, as embryos are used instead of adult/juvenile fish. We are developing a strategy for incorporating the FET into the threshold approach to provide a means for reducing the number of fish used in acute aquatic toxicity testing. This strategy is building on extensive earlier work and drawing upon the work of three individual efforts.A new database containing acute toxicity data for adult/juvenile fish, fish embryos, Daphnia and algae has been constructed to analyse how the FET can be incorporated into the threshold approach. The applicability domain of the FET will be clarified, and the uncertainties of the FET (focusing on metabolism) will be compared to the uncertainties of the AFT for the protection of the aquatic ecosystem. On this basis, a concept for defining acceptance criteria for the new approach will be proposed. A recently published report by the European Chemicals Agency on the use of the FET for REACH is being considered. This poster presents progress to date. The views, conclusions and recommendations are those of the authors and do not necessarily represent the policies or positions of the organisations to which the authors are affiliated.

RP212 Methodology to visualize skeletal defects in inland silverside Menidia beryllina exposed to levonorgestrel

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The inland silverside Menidia beryllina is zooplanktivorous and inhabits rivers and estuaries along the East, West, and Gulf coasts. Their high sensitivity to chemicals, small body size, short generation time, and small-batch fecundity make them an ideal toxicological study species. Toxicological studies have been conducted on abnormal skeletal development of the inland silverside after exposure to certain chemicals; however, to the best of our knowledge no studies have used calcein staining to visualize the resulting abnormalities in this species. Calcein (CAS 1461-15-0) is a fluorochrome dye that binds to calcified structures. When excited with blue light (of ~488 nm), it emits a green fluorescence (~520 nm). Calcein has been successfully used in aquaculture to non-invasively mark juvenile fish, and to examine skeletal development in the model zebrafish. The goal of this study was to determine whether calcein has the potential to be a useful tool to better understand the effects of environmental contaminants on bone development in M. beryllina. Previous work in our laboratory demonstrated that skeletal abnormalities occur after exposures to ng/L concentrations of endocrine disrupting chemicals (EDCs). We therefore designed a series of tests to elucidate the best methodology for calcein staining of M. beryllina skeletal structures in order to examine such abnormal skeletal development. After immersing live larvae in 2-g/L of calcein for 7 minutes, calcification of major bone structures were successfully seen between 9-21 days post fertilization. We subsequently exposed M. beryllina embryos to environmentally relevant concentrations of levonorgestrel from 5 hpf to hatch (168-192 hpf). Fish were immediately stained with calcein and imaged using a confocal microscope to quantify skeletal abnormalities in the craniofacial region. This method will be useful for better understanding skeletal abnormalities in larval stages of this model fish species resulting from exposure to environmentally relevant concentrations of EDCs.

RP213 Strategies for Characterizing Species Sensitivity in Screening Level Assessments for Data Poor Industrial Chemicals

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The EPA Office of Pollution Prevention and Toxics (OPPT) is responsible for implementing the Toxic Substances Control Act (TSCA) which regulates industrial chemicals in the US. Under TSCA, the notifier is not required to conduct any “new” ecological or human health testing before submitting a PMN and, thus, many substances are submitted with little or no experimental data. To conduct risk assessments with little or no data, OPPT has developed a strong knowledge base in quantitative structure activity relationships (QSARs) based on available laboratory data. These QSARs represent a collection of curated experimental data that are categorized by chemical class and taxa for the standard aquatic toxicity endpoints used in screening level risk assessments. The endpoints represent hazard potential to three levels of taxa including vertebrates (fish), invertebrates, and algae. The species included in the curated data sets for each taxa are those allowed under the standard test guidelines (i.e., OCCSP and OECD Guidelines). Although many species are represented in a QSAR, often data available for a chemical are for a single species within the taxa. In the new chemicals program, potential variability in toxicity between species is addressed using a standard set of assessment factors that are applied to the estimated or measured effect levels. However, if a QSAR class is sufficiently robust (i.e., Neutral Organics, Esters, Phenols, Aliphatic Amines), plotting effect levels against Log Kow can inform on the class-specific species variability represented by the spread of data across the regression lines. USEPA is investigating the usefulness of class specific assessment factors developed from this demonstrated spread of data to better account for species variability within a specific class and taxa in conjunction with updates to the USEPA

ECOSAR program as more robust data sets become available. For each chemical class where sufficient data are available, the data will be plotted and the spread of data around the trend line will be depicted and discussed. The views expressed in this abstract are those of the authors and do not represent Agency policy or endorsement.

RP214 Passive Dosing Functional Water Solubility and Implications for Aquatic Toxicity Testing

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Over the last few years techniques to introduce and maintain stable saturation concentrations of substances and mixtures for aquatic toxicity tests using passive dosing have been reported. These techniques typically utilize silicone tubing or solid silicone O-rings as the reservoir for the test material, and the dosing in water follows partitioning equilibrium theory. The technique also provides an opportunity to quantify the saturation water solubility of substances that are difficult to measure using standard water solubility methods. However, caution must be exercised for multi-component substances or mixtures as minor constituents or impurities may be the most water soluble components and may introduce analytical quantification issues, as well as issues for aquatic toxicity testing. Examples of solubility experiments of multi-component substances using different passive dosing techniques, along with discussions of analytical challenges and implications for aquatic toxicity testing using these techniques will be presented.

RP215 Development of Culturing and Rearing Methodology for Ephemeroptera Taxa

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Mayfly taxa may be more sensitive to aquatic pollutants than standard test organisms used to determine anthropogenic effects on aquatic ecosystems. Mayflies, however, are not frequently used in toxicity testing and few studies have addressed culture methods for mayfly taxa. Additionally, while larger mayflies can be field collected for use in bioassays, the most sensitive stage may be newly hatched individuals and the relative sensitivity has not been established for most species. To answer these questions, and to provide organisms of consistent health for use in toxicity testing, mayflies are being reared in the laboratory. Culture methods for rearing larval mayflies to emergence, collecting viable eggs sexually and parthenogenetically, and rearing eggs to hatch have been developed in the Marshall University Lab. Currently, evaluations are underway to determine if hatch rate can be manipulated in developing egg clutches to synchronize hatching and be more for conducive for use in toxicity testing. Further development of the methods in order to conduct native mayfly toxicity testing is dependent on a suitable food source being established for cultured mayfly nymphs. The objective of the study described herein was to investigate adequate food alternatives that will lead to a minimum of 80% survival in juvenile mayflies the first 48 hours after hatch. This endpoint was selected because standard toxicity testing requires an 80% survival within the control organisms for the test to be considered valid. Tests were conducted using a variety of diets incorporating laboratory cultured algal alternatives and natural and artificial foods options such as ground fish food, yeast-cereal leaves-trout chow and leaf disks. Diet treatments were given to individual nymphs in separate chambers throughout the course of a 36-day test. Mortality rates from preliminary testing with 11 feeding treatments were used to narrow food types to the ones yielding the best results for further testing. Success was evidenced by the growth and development of the nymphs, measured as number or exuvia shed and survival with the goal of 80% survival in the first 48 hours after hatch. Two of the four feeding treatments provided over 80% survival of newly hatched mayfly nymphs in 48 hours. The other two provided over 70% survival in 48 hours. A diet of laboratory cultured Navicula sp. and leaf disks supported the greatest survival over the 36 day study.

RP216 A comparison of the survival of Leptocheirus plumulosus and Corophium volutator in the 10-day Sediment Toxicity Test using Formulated Sediment

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Synthetic based fluids slated for use in the Gulf of Mexico must pass the 10-day Sediment Toxicity test, EPA Method 1644, using L. plumulosus. The Offshore Chemical Notification Scheme requires several different types of testing to be completed on chemical components designated for use in the exploration, exploitation and associated offshore processing of petroleum on the UK Continental Shelf. If the chemical or component is a “sinker”, has a Log Pow >4, or is in any other way known to adsorb to particles or end up in the sediment, or contain surfactants, a sediment re-worker test using C. volutator must be performed. This study evaluates the performance of L. plumulosus and C. volutator using EPA Method 1644. The toxicant used was a C16/18 IO which is also used as the standard reference material for the test method. Six treatments were prepared with formulated sediment, five test substance concentrations and a negative control. Twenty organisms were exposed to each test treatment for 10 days. The endpoint for each test was survival.

RP217 The Uncertainties Associated with Detection and Quantification of Intersex (Testicular Oocytes) in Fish

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Over the last two decades, several teleost fish species have been detected with testicular oocytes (TO), resulting in a variety of techniques to characterize the occurrence and severity of the condition. Primary methodological differences relate to the orientation of histologic sections (transverse vs. longitudinal), number and spatial distribution of histological sections observed, and quantification of TO severity (direct enumeration of observed TO vs. application of a ranking system to describe number, relative proximity and, where appropriate, developmental stage of oocytes). Resulting estimates of TO prevalence and severity will often differ in meaningful ways so care must be taken to recognize these distinctions when comparing study results and when designing studies. To address the benefits and caveats of each method, largemouth bass Micropterus solamoides testes were subjected to transverse, longitudinal, and biopsy sectioning (biopsies collected via laparoscopy, a non-lethal surgical technique). It is paramount to understand the implications of each available technique to collect tissue (lethal vs. non-lethal) and each subsequent methodological step when designing an investigation to ensure its comparability to findings in the scientific literature. With few exceptions, correlations of TO metrics should be investigated at the population level rather than the individual level due to variability in sub-samples and ability to detect TO. Data was analyzed across sample sites by comparing TO prevalence and severity from each step-section singly, and across all step-sections, to determine the variability based on level of observational effort and method employed. Detailed results and implications will be discussed.

RP218 Development of quantitative structure activity relationship models to predict the toxicity of fluorinated chemicals to aquatic organisms

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Over the last 12 to 15 years, a concentrated effort has been made to identify and quantify fluorinated compounds in the aquatic and terrestrial environments. Initially, these programs focused on perfluoroalkyl sulfonic acids (PFSAs) and perfluorocarboxylic acid (PFCAs). However, as analytical methods and instrumentation have improved, the number of fluorinated classes that have been identified in environmental matrices has increased dramatically to include fluorotelomers, polyfluorinated phosphate esters (PAPs) and perfluorophosphonates (PFPAs). In contrast, the assessment of the potential risks these fluorochemicals pose biota has progressed much more slowly. With the exception of perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) few fluorochemicals have been tested sufficiently to support many regulatory needs including the support of pre-manufacturing notices, water quality criteria and ecological risk assessment. One means to address this lack of data is to develop quantitative structure activity relations (QSAR) that can be used to screen and rank the fluorochemicals as to their potential environmental risks. Studies have shown that within different classes of fluorochemicals, the toxicity to aquatic organisms tend to increase with the length of the fluorinated carbon chain. However, branching, inclusion of heteroatoms and differences in terminal functional groups in the non-fluorinated portion of theses compound can also alter toxicity making it difficult to predict toxicity across the various classes of fluorochemicals. To address this issue, a systematic evaluation of available ecotoxicological data for fluorochemicals was conducted. For fluorochemicals with adequate toxicological data, molecular descriptors were determined that included topographical, geometrical, electrostatic, and quantum chemical endpoints. QSAR models were developed using multiple linear regression (MLR) and principal component analyses (PCA) methodologies. Preliminary results from these models indicate that reasonable estimates of acute toxicity to several species can be made using this type of approach. Results from these analyses will be presented and uncertainties and areas of future research will be discussed.

RP219 Mosquitofish as a model for endocrine disruption; the impacts of exposure to arsenic on reproductive function and development

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A wide range of contaminants can interfere with hormonal regulation in vertebrates. These endocrine disrupting compounds (EDCs) are of high relevance for human and wildlife health. Endocrine signaling controls essential physiological processes, which have an impact on an individual's health, such as growth and development, stress response, and ultimately reproduction and population development. The metalloid, arsenic, is known to cause various detrimental health problems including cancer and non-cancer illnesses and has a regulatory limit of 10 ppb in drinking water. At low environmentally relevant levels, arsenic has been identified as an EDC that disrupts normal function of thyroid, adrenal, and gonadal axes. Arsenic has been shown to inhibit androgen receptor (AR) recruitment to AR target genes in sertoli and prostate cells. For males, EDCs that have anti-androgenic activity may lead to alterations in genetic signaling pathways, hormonal profiles, and subsequently, secondary sex characteristics. To determine potential health outcomes from arsenic exposure, juvenile male mosquitofish (Gambusia affinis) were collected and exposed to sodium arsenate (0 ppb, 0.7 ppb, 7 ppb, 74 ppb) in the lab for 30 days. Morphological endpoints including body length, body mass, gonopodium somaticindex (GSI), and gonopodial lengths, indicative of androgen-dependent secondary sex characteristic development, were evaluated. Total length, standard length, condition factor, and mass did not differ among treatment groups. Using geometric morphometric analysis, we found that body shape was significantly narrowed in the 74 ppb group. GSI and gonopodial lengths were significantly lower, showing a reduced growth effect, in the 7 ppb and 74 ppb treatment groups. However, differences in the 4:6 ratio among treatments were not significant suggesting that the overall fin growth is being inhibited. Our results suggest that arsenic exposure below current regulatory limits affects general growth and reproductive development in a sentinel fish species. Future work will determine whether molecular reproductive endpoints can also act as biomarkers for arsenic exposure in Western mosquitofish under both laboratory and field conditions.

RP220 Potential Aquatic Toxicity of Petroleum Biodegradation Metabolites in Groundwater Samples from Fuel Release Sites

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Petroleum hydrocarbons biodegrade in the environment and are converted to polar (non-hydrocarbon) metabolites. These metabolites are extracted from groundwater samples and quantified as Total Petroleum Hydrocarbons (TPH) unless a silica gel cleanup (SGC) step is applied to the sample extract prior to the TPH analysis. The polar metabolites can be separated from the non-polar hydrocarbons by incorporating the SGC step; however, some regulatory agencies are hesitant to adopt this method, citing the unknown nature and toxicity of these complex mixtures. Using state-of-the-art non-targeted GCxGC-MS analyses of groundwater samples from historic fuel release sites, we tentatively identified thousands of polar metabolites representing many distinct structural classes of chemicals including acids/esters, alcohols, phenols, ketones, and aldehydes. The potential toxicity of polar metabolites to freshwater aquatic organisms has been investigated as part of this study. Briefly, upgradient (representing local background conditions) and downgradient groundwater samples were collected from several representative biodegrading fuel release sites, and submitted to a contract laboratory for chronic aquatic toxicity testing for freshwater organisms using EPA test methods 1000 (Fathead Minnow), 1002 (Daphnid) and 1003 (Green Algae). Results show that aquatic toxicity of groundwater samples from fuel release sites are primarily due to background water quality, and not from biodegrading petroleum metabolites. Overall, the complex mixtures of polar metabolites, at concentrations typically found at petroleum release sites, are unlikely to pose a significant risk to aquatic life.

RP221 Dredging and seasonality effects on nutrient concentrations and ecoenzymatic activity in agricultural drainage ditch sediments

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Agricultural drainage ditches are conduits between production acreage and receiving aquatic systems. Often overlooked for their mitigation capabilities, agricultural drainage ditches provide an important role for nutrient transformation via microbial metabolism. Variations in ecoenzyme activities have been used to elucidate microbial metabolism and resource demand of microbial communities to better understand the relationship between altered nutrient ratios and microbial activity in aquatic ecosystems. Two agricultural drainage ditches, one in the northeast portion of the Arkansas Delta and the other in the lower Mississippi Delta, were monitored for a year. Sediment samples were collected prior to each ditch being dredged (cleaned), and subsequent post-dredging samples occurred as soon as access was available. Seasonal samples were then collected throughout a year to examine effects of dredging and season on nutrient concentrations and ecoenzymatic activity recovery in drainage ditch sediments. Significant differences in phosphorus concentrations were noted at almost every location and sediment depth, primarily between the pre-dredge and post-dredge samples. Dredging decreased sediment phosphorus concentrations between 33-51%, depending on sample location and depth. Likewise, ecoenzymatic activity was significantly decreased after dredging in most samples. Fluorescein diacetate hydrolysis activity in one ditch decreased 56-67% after dredging. Many sample sites also had significant phosphorus and ecoenzymatic activity differences between the post-dredge samples and the year-long follow-up samples. Results indicate microbial metabolism in dredged drainage ditches may take up to a year or more to recover to pre-dredged levels. Likewise, while sediment nutrient concentrations may be decreased through dredging, runoff and erosion events over a year tend to quickly replenish nutrient concentrations in sediments. Understanding microbial metabolism and nutrient dynamics within agricultural drainage ditches is a first step toward addressing issues of nutrient enrichment in aquatic receiving systems, such as the Gulf of Mexico.

RP222 Endocrine disruptors and parasitism: what effects on the sex behaviour of the amphipod Gammarus pulex ?

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Endocrine disruptors (EDCs) are well known to disrupt the development and the reproduction of exposed organisms. Although this point has been studied in vertebrate models, the limited knowledge of the endocrine system of invertebrates makes the evaluation of EDCs effects difficult. However, invertebrates represent the major part of aquatic ecosystems, such as amphipods Gammaridea, which are crucial for their functioning (e.g. litter degradation, food resource). Moreover, gammarids are hosts of parasites such as vertically-transmitted microsporidia (microsporidia VT), which could be confounding factors in assessment of EDC effects. Indeed, some microsporidia VT could have endocrine effects by their own present in the host, since it was observed for example, a feminisation of juvenile males, which become phenotypic females. This study aimed to assess the effects of the ethinylestradiol (EE_2) and the cyproterone acetate (CPA), EDCs commonly studied in vertebrates, and the presence of microsporidia VT, on the sex behaviour and the fitness of the freshwater amphipod Gammarus pulex during a 30 days-exposure. Several parameters were recorded such as: the time of amplexus formation, the mate-guarding time, and numbers of spermatozoa and oocyte produced. Results revealed that less amplexus were formed when G. pulex were exposed to EDCs, especially to EE_2. However, G. pulex exposed to EE_2 0.05 $\mu g.L^{-1}$ took less time to form amplexus than controls, whereas G. pulex exposed to EE_2 5 $\mu g.L^{-1}$ took longer time. About parasitism, when exposed to EE_2 0.05 $\mu g.L^{-1}$, parasitized G. pulex were more in amplexus than respective control. Moreover, whatever the exposure condition, the percentage of parasitized females in amplexus were higher than in controls. Finally, no significant effect of EDCs was observed on the spermatozoa and oocyte in unparasitized gammmarids, but weaker spermatozoa counts were measured in presence of microsporidia VT. Under CPA stress, parasitized males produced significantly less spermatozoa than respective controls. Results highlight that EE_2 and CPA caused low effects on the sex behaviour and the fitness of G. pulex, unlike microsporidia VT presence, which decrease the amplexus time formation, increase the percentage of females in amplexus, and reduce the spermatozoa production, in stress conditions. Thus, results suggest that the parasite presence affected the gammarids behaviour in order to tend to increase its own fitness (e.g. transmission).

RP223 Infradian control of Daphnia magna molt cycle and its disruption by nitric oxide

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Internal molecular clocks control influential biological processes and environmental cues aid in the regulation of the timing of these rhythms. Environmental chemicals may disrupt these rhythms resulting in profound adverse consequences. The molting in arthropodsis initiated by the synthesis and inactivation of 20-hydroxyecdysone. Disruption in the timing of the 20-hydroxyecdysone peak can adversely impact both growth and reproduction. We hypothesized that the overall duration of the molt cycle in crustaceans is dictated by a cascade in the sequential expression of several nuclear receptors and cytochrome p450 (CYP) enzymes. Further, we propose that pulses in nitric oxide control the availability of one of these proteins, HR3, and as such, acts as a fine-tuner of the timing of the molt cycle, perhaps in response to environmental cues. Lastly, we propose that exposure to exogenous sources of nitric oxide such as nitrate and nitrite can disrupt the timing of the molt cycle resulting in adverse impact on growth and reproduction. Analysis of mRNA expression of relevant proteins during the molt cycle of adult daphnids (Daphnia magna) revealed the sequential production of E75, HR3, FTZ, 20-hydroxyecdysone-synthesizing CYPs, and 20-hydroxyecdysone-inactivating CYP. Early exposure to a nitric oxide donor sodium nitroprusside (SNP) resulted in a significant increase in the magnitude and delay in timing of peak expression of FTZ and CYP18a1 mRNA expression as well as a delay in molting. CYP18a1 is the CYP enzyme responsible for inactivation of 20-hydroxyecdysone. We conclude that exposure to an exogenous source of nitric oxide caused excess availability of free HR3 protein resulting in excess expression of downstream proteins. This may ultimately result in a prolongation of the duration of the molt cycle with negative consequences on growth and reproductive rates.

RP224 The Effects of Aquatic Nitrate Pollution on Zebrafish (Danio rerio) Embryo Respiration

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Nitrogenous compounds naturally cycle through the air, aquatic systems, and soil. Global nitrogen has been increasing in quantity due to anthropogenic sources, whether intentional or unintentional, that include agricultural fertilizer, fossil fuel combustion, and exhaust gasses. Excess nitrogen in agricultural fertilizers can leach into groundwater and presents the potential for contaminating drinking water and affecting aquatic wildlife. To assess the effects of nitrate pollution, zebrafish embryos are used as a model organism. This research seeks to understand nitrate's function in zebrafish embryo respiration as measured by oxygen consumption rates. Oxygen consumption rates (OCR) are determined using a novel approach Seahorse Bioscience XF^{e}96 extracellular flux analyzer specially designed for use with zebrafish embryos. OCR analyses were performed for five nitrate treatment concentrations that included 0, 5, 10, 50 and 100 mg/L NO_3-N with both sodium nitrate or potassium nitrate. To separate the effects of nitrate from the carrier ion, whether sodium or potassium, OCR analyses were also performed for four vehicle control treatment concentrations equivalent to the amount of sodium or potassium within each of the nitrate treatments with sodium chloride or potassium chloride, respectively. Zebrafish embryos were chronically immersed in the respective treatment concentrations for 24 hours post fertilization before 75 minutes of OCR measurements were recorded. OCR measurements were averaged and normalized to a 0.05µM FCCP positive control treatment. Dunnett's multiple comparisons test following a significant one-way ANOVA was used to compare treatment OCR values to control OCR values. Sodium nitrate treatments of 50 and 100 mg/L NO_3-N, as well as the corresponding vehicle controls for each treatment significantly reduced OCR compared to control. Potassium nitrate treatments of 10 and 50 mg/L NO_3-N, as well as the 50 mg/L and 100 mg/L vehicle control treatments significantly reduced OCR compared to control. Significant vehicle control OCR analyses represent an often-overlooked aspect of laboratory nitrate research, as the carrier ion is often not examined for its effects. In addition to immersion OCR analyses, injection of potassium and sodium nitrate through the zebrafish embryo chorion will be investigated for its effects on embryo respiration and to investigate how different routes of laboratory nitrate exposure may impact embryo respiration.

RP225 Development of an in vivo anti-androgenic activity detection assay using fenitrothion (MEP) in Japanese medaka (Oryzias latipes)

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The effects of endocrine disruptors, such as anti-androgenic chemicals, on aquatic environments, has caused increasing concern in recent years. Currently, the method used to screen chemicals for anti-androgenic activity is called the androgenised female stickleback screen (AFSS), and it was established by the Organization of Economic Cooperation and Development (OECD) in 2011 using the stickleback. However, screening assays have not been developed in other fish species. In this study, we present the detailed description of the development and evaluation of a screening method for anti-androgenic activity in chemicals by utilizing Japanese medaka (Oryzias latipes). Using this model, we focused on the number of papillary processes as an indicator of a chemical's anti-androgenic activity. Thus, at 35 days post fertilization, medaka were exposed to fenitrothion (MEP), an anti-androgenic compound, for 28 days. In the control group, the formation of papillary processes was observed in XY medaka, but not in XX medaka. However, after MEP exposure, the number of papillary processes were significantly decreased in a dose-dependent manner in XY medaka; in the 300 µg/L concentration group, four out of 11 XY medaka showed no papillary processes even if there were no significant effects on total length and wet body weight compared with the control group. Our results indicate that the number of papillary processes in Japanese medaka can be used as an indicator of anti-androgenic activity and that this model may prove useful as a chemical screening method.

RP226 Combined effects of endocrine disruptors and parasitism on the molting process of the invertebrate amphipod Gammarus pulex?

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Endocrine disruptors (EDCs) are known to modify population dynamics of exposed organisms. In amphipod Gammaridea, growth and reproduction are linked to the molt process; hence, EDCs could affect populations through molt disruptions. However, Gammaridea are hosts of parasites, such as vertically-transmitted microsporidia (microsporidia VT), which could be confounding factors in assessment of EDC effects. Indeed, some microsporidia VT could have endocrine effects by their own present, as it was observed a feminization of juvenile males (e.g. disruption of androgen gland). This study aimed to assess the effects of ethinylestradiol (EE_2), cyproterone acetate (CPA), 4-hydroxytamoxifen (4HT) and 17α-methyltestosterone (17MT), and the presence of microsporidia VT, on the 20-hydroecdysone concentration (20-HE, molt hormone) and the chitobiase activity, enzyme involved in the degradation of the old cuticle, in Gammarus pulex males and females, after a 96h laboratory exposure. Results revealed that only EE_2 and CPA impacted the 20-HE concentration in uninfected males, and no effects were observed in parasitized males. In uninfected females, no effects were measured, but 20-HE concentrations increases were observed after EDC exposures. For chitobiase analyses, no differences were observed in uninfected males after EDC exposures, but a trend to increased activity was measured in infected males. The opposite result was highlighted in females, since significant increases of chitobiase activity were measured in uninfected females exposed to 4HT and 17MT, and trends to increased activity were observed in infected females. Results also underlined no difference between the 20-HE concentration in uninfected and infected individuals. However, parasitized males and females had lower chitobiase activities than uninfected individuals. The study shown that EDCs affected the 20-HE concentrations and the chitobiase activity of G. pulex, differently according to the gender and parasitism. This suggest that EDCs, proved in vertebrates, could also disrupt the population dynamic of invertebrates such as G. pulex. Moreover, EDCs effects, more important on the 20-HE concentrations than on the chitobiase activity, suggest that EDCs disturb the molt mechanism by its first stages. Finally, results revealed low chitobiase activities in parasitized G. pulex, which could leading to a slower molt, thus a slower reproduction, contrasting with the parasite fitness (transmission by reproduction).

RP227 Inhibition of swim bladder inflation in Japanese medaka (Oryzias latipes) larvae in the absence of an air-water interface

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Physostomous fish maintain their embryonic connection between the gut and the gas bladder via a pneumatic duct, whereas this connection degenerates in physoclistous fish resulting in a closed swim bladder. In physoclistous larval fish, the pneumatic duct only exists for a short period of time before it regresses. Therefore, swim bladder inflation must occur by ingesting air at the water surface during this time. Japanese medaka (Oryzias latipes) are physoclistous fish and were used to assess the time needed to inflate the swim bladder before the closure of the pneumatic duct. Control Japanese medaka larvae have a swim bladder inflation rate of 99.6 +/- 1.4 % within 3 days of hatching. Covered larvae, denied an air-water interface (4-8 days), were completely unable to inflate their swim bladders post hatch until they were uncovered. Furthermore the amount of time post hatch which the larvae were denied an air-water interface had a significant reduction on their ability to inflate their swim bladders when they were exposed to an air-water interface. This effect was time dependent with significant effects being observed from 4-8 days covered. The longer the fish were denied an air-water interphase the less fish were able to inflate their swim bladders when uncovered. A significant increase in mortality was also observed in all the treatments that were covered for four or more days. These results indicate that access to an air-water interface is crucial for the initial inflation of Japanese medaka. Inhibition of swim bladder inflation of larval Japanese medaka has the potential to cause population level effects by reducing growth and survival.

RP228 Effects of Temperature on the Endocrinology of Smoltification in Juvenile Rainbow/Steelhead Trout (Oncorhynchus mykiss)

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The San Francisco Bay Delta is experiencing seasonally warmer waters and salt water intrusion into historically freshwater ecosystems due to climate change. Juvenile endangered Steelhead Trout (Oncorhynchus mykiss) inhabit this system from juvenile development through the smoltification process. It is possible that juvenile fish experience premature hypersaline acclimation due to seawater intrusion (PHA), and the effects of increased temperature and PHA on pre-smolt Steelhead are unknown. Rainbow trout (Oncorhynchus mykiss) were used as a genetic model for Steelhead, since they are the same species. Juvenile fry were exposed to 110C, 16.40C and 190C temperatures (n=12) for two weeks and then challenged for 24 hours to 32ppt seawater. Estradiol-17? (E2), cortisol, triiodothyronine (T3), thyroxine (T4) levels were measured in blood/plasma or whole animal homogenates using Enzyme Linked Immunnosorbent Assays. Gill Na+/K+ ATPase mRNA levels were measured using qPCR. Preliminary results show 50% survival and a hepatosomatic index (HSI) of 0.89% in fish exposed at the highest temperature compared to an average HSI of 1.24% in fish exposed to the optimal temperature. Ongoing studies will explore the impacts of PHA and pesticides on smoltification and osmotic stress in multiple life stages of salmonids. This material is based upon work supported by the Delta Stewardship Council Delta Science Program.

RP229 Effects of ALCOA Solid Waste Leachate on Water Quality of the Yadkin River: A Badin, NC Case Study for Ecotoxicology's Role in Social Justice

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Social justice is one of the three pillars of the Sustainability movement spreading across academic institutions worldwide. Here we introduce an aquatic ecotoxicology/social inequality case study in which an underprivileged community in Badin, NC is forced to contend with toxic leachate from one of the only retired ALCOA plants not designated as a USEPA Superfund site in the US. Site designation under Superfund provides funding for and oversight of cleanup activities. Often problems that exist in lower income communities are overlooked by political and/or government entities and therefore continue on indefinitely. The Badin Works facility was operated as an aluminum smelting plant by Aluminum Company of America (ALCOA) from 1916 to 2007. During that time cyanide, fluoride, PCBs, PAHs and other toxins, including arsenic, were released through ALCOA's 13 outfall pipes into Badin Lake and into Little Mountain Creek, which is listed as impaired by NCDENR due to poor bioclassification. Additionally, hazardous materials were buried in unlined landfills at 44 known locations. For the last 3 years the Appalachian State University Aquatic Ecotoxicology Lab has partnered with the Duke Environmental Law and Policy Clinic, Badin Concerned Citizens, and the

Yadkin RiverKeeper to analyze water, sediments, fish and macroinvertebrates from Badin to support elevation of the contaminated Badin sites to Superfund status. Studies have consistently shown elevated levels of cyanide in water and soil (22 ppb and 1.8 ppb, respectively) and fluoride in water (26 ppm). Also, elevated levels of PCB's in soil (53 ug/kg Aroclor 1248) and fish tissue (as high as 340 ug/kg of Aroclor 1248) and PAH levels in soils (29 ug/kg Benzo(a)pyrene) were reported. Although results of NC DWQ benthic macroinvertebrate bioindicators have consistently shown excellent water quality, the fish community IBIs have shown declines from Good/Fair to Poor over the three years of sampling. Because our data supported the Badin community's request for Superfund status, in November 2014 the USEPA agreed to conduct a preliminary assessment for Superfund status of the retired ALCOA smelter site. Through a diverse partnership that strategically combined complementary resources we have made great strides towards the goal to correct this long overlooked issue.

RP230 A short summary of environmental fate (biodegradability) and ecotoxicity data for ENORDET O-series surfactants

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A host of chemical enhanced oil recovery (cEOR) technologies are currently being evaluated to extend the life and maximise production of oil from new and existing reservoirs. Shell Chemicals is developing two general classes of surfactant for enhanced oil recovery: alcohol alkoxy sulfates (ENORDET J- and A-series) and internal olefin sulfonates (ENORDET O-series). These products are currently being evaluated in pilot projects around the world to demonstrate the potential of surfactant-based cEOR technology. Shell has commissioned a range of baseline environmental fate and ecotoxicological effect studies on selected products from within the ENORDET surfactant portfolio to assist and comply with global regulatory notification and registration requirements. The objective of this poster is to provide an overview of the experimental environmental fate and effects data currently available for ENORDET O-Series surfactants and how this compares with analogous classes of anionic surfactants (e.g. detergent range surfactants). The challenges faced with generating scientifically robust and relevant environmental test data for ENORDET surfactants along with potential opportunities for future research and testing will also be addressed.

RP231 Comparative Toxicity Of Sodium Carbonate Peroxyhydrate To Freshwater Organisms

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Episodic exposures of sodium carbonate peroxyhydrate (SCP) algaecides in aquatic systems prompt the need to understand innate sensitivities of aquatic organisms to exposures of the active ingredient, hydrogen peroxide. Measurements of the relative sensitivities of algal, invertebrate, and vertebrate species to laboratory exposures of hydrogen peroxide can be used to interpret potential risks from SCP applications in aquatic environments.The objective of this study was to measure and compare responses of a cyanobacterium (Microcystis aeruginosa), a eukaryotic alga (chlorophyte Pseudokirchneriella subcapitata), invertebrates (microcrustacean Ceriodaphnia dubia and benthic amphipod Hyalella azteca), as well as the vertebrate fathead minnow (Pimephales promelas) to exposures of hydrogen peroxide as SCP. Hydrogen peroxide exposures were confirmed using the I_3^- method. Measured relative sensitivities of organisms exposed to SCP were compared with published toxicity data to provide context for other algaecides and herbicides (e.g. copper formulations, endothall, and diquat dibromide). Algal responses (cell density and chlorophyll a concentrations) and animal mortality were measured after 96-h aqueous exposures to SCP in laboratory-formulated water to estimate EC_{50} and LC_{50} values, as well as potency slopes. M. aeruginosa was more sensitive to hydrogen peroxide as SCP (96-h EC_{50}: 0.9-1.0mg L^{-1} H_2O_2) than the eukaryotic alga P. subcapitata (7-d EC_{50}: 5.2-9.2mg L^{-1} H_2O_2), indicating potential for selective use of SCP exposures to control prokaryotic algae. For the vertebrate and invertebrate organisms evaluated, measured 96-h LC_{50} values ranged from 1.0 to 19.7mg L^{-1} H_2O_2. C. dubia was the most sensitive species, and the least sensitive species was P. promelas, which is not likely to respond to concentrations of hydrogen peroxide as SCP affecting cyanobacteria (e.g. M. aeruginosa). Based on information from peer-reviewed literature, exposures of other algaecides could cause similar differential responses of prokaryotic algae compared to eukaryotic algae. Of the algaecides compared, exposures of SCP can mitigate risks associated with noxious cyanobacterial growths (e.g. M. aeruginosa) while minimizing potential risks for other species (e.g. P. promelas).

RP232 Effects of Industrial Activity on Trace Element Accumulation, Community Composition, and Population Structure of Lotic Dragonfly Nymphs

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Evaluating impacts of environmental contamination is an essential component of assessing a stream's need of and potential for restoration or enhancement. Presented results are a portion of an overall integrated assessment of the hydrology, geomorphology, biology, and contaminant accumulation in Coastal Plain streams on the Savannah River Site, SC, USA. We used 7 genera of dragonfly nymphs as biomonitors of contaminants entering the aquatic food web and impacts of excessive stormwater runoff. Two reference streams were compared to 3 streams receiving varying amounts of stormwater runoff and effluents with two streams being more severely scoured. Dragonfly nymph generic richness and diversity were reduced in the two most scoured streams as more sensitive species were missing and others reduced in relative abundance. Life history differences between disturbed and reference streams also included missing or poorly represented cohorts based on size frequency analyses. Additionally length-weight relationships differed between disturbed and reference streams in some genera. Variation in element accumulation among genera clearly exceeded variation within genera suggesting that genus is a reasonable taxonomic level for both spatial and taxonomic comparisons. Patterns of trace element accumulation in biota tended to be element and taxa dependent, but patterns of elements frequently accumulating to higher concentrations in the disturbed sites were evident. Some elements such as Mn, Co, Ba, and Ni showed particular taxonomic variability that was compounded in disturbed sites. Boyeria, Hagenius, Macromia, and Erpetogomphus accumulated higher concentrations of these elements than Dromogomphus, Gomphus, and Cordulegaster. In contrast, Se and As tended to accumulate higher in Dromogomphus and were elevated in most genera at the two most disturbed sites. Al, V, and Fe were also consistently elevated in all three disturbed sites in most genera. Influences of body size, trophic level, carbon sources and surface area to mass ratios on trace element accumulation were evaluated. Overall, dragonfly nymph community and population structures were altered in the two most scoured streams and several trace elements were accumulating to higher levels in all three disturbed systems.

RP233 Spatial Analysis Methods for Assessing Sediment Injury in Natural Resource Damage Assessment

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Natural Resources Damages Assessments (NRDAs) for injury to sediment typically rely on the use of Habitat Equivalency Analysis (HEA) as a means of determining the scope of compensatory mitigation necessary to offset lost services due to a release. HEA, in turn, is a tool for estimating the spatial dimension of service loss over time and expresses service losses and gains in units of service-acres-years (SAYs) defined as the level of ecological services provided by one acre of sediment habitat over a one year period under baseline conditions. Application of HEA involves two fundamental steps: 1) development of quantitative relationships between concentrations of one or more constituents in sediment and inferred ecological service loss, and 2) use of geographical information systems to conduct spatial analysis of sediment chemistry to determine service losses by unit area (SAYs)—and the outcome of any HEA analysis is highly sensitive to the assumptions inherent in each of these steps. This presentation compares and contrasts the effects of two spatial analysis methods (Thiessen polygon and Inverse Distance Weighting) using a real-world data set, presents strengths and weaknesses of the two approaches, and discusses ways in which uncertainty can be minimized.

RP234 Bioaccumulation and toxic effects of resin acid for microalgae and microcrustaceans

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Pulp and paper production process generate a great variety of compounds that can cause adverse effects on several organisms. The resin acids such as abietic acid are among those compounds that exhibit high resistance to chemical and biological degradation that associated with their hydrophobic nature can exhibit increased toxicity due to bioaccumulation effect. This study aimed to evaluate the toxic effects of different concentrations abietic acid (10 ug/L and 50 ug/L) in organisms from different trophic levels (microalgae – Scenedesmus subspicatus and microcrustacean – Daphnia magna) and bioaccumulation potential for both. Bioassays with Daphnia magna were carried out according to OECD – Test No. 211 (OECD, 2012). Bioassays were performed with Scenedesmus subspicatus according to ISO 8692 (ISO 2012). Abietic acid exhibited no toxicity for microalgae. The results showed that daphnideos that were fed with algae (106 células.mL-1) that grew up in different abietic acid concentrations showed a reduction in the number of pups compared to controls, a reduction of up to 83%. Additionally, significant morphological changes of daphnideo spine were observed in all tested abietic acid concentrations, and it was absent in controls. The presence of males was also observed, especially for higher concentration of abietic acid. Chromatographic analysis by GC/ MS were able to detect abietic acid in microalgae cell at 3h-exposure period and no abietic acid was detected in the 24 and 48h-period, although toxicity has been high in the same period. In conclusion, preliminary results showed that the algae were able to bioaccumulate abietic acid/or a metabolite and the biomagnification phenomenon can be observed along the food chain. Support: Fapesc/CNPq

RP235 Comparison of Statistical Approaches for Count Data from Ecotoxicity Studies

J.W. Green, DuPont / Applied Statistics Group

There are two types of count data of that arise in ecotoxicity studies. Quantal data is based on a fixed number of subjects that are exposed and the interest in is the number of those subjects that respond, e.g., die or develop a specific type of lesion or condition. The type of count data to be discussed here can take on a wide range of possible values that vary by individual subject. Examples include the number of eggs laid and the number of live young produced. Typically, count data have been analyzed as though they are continuous, often after a square-root transform. Advances in statistical methodology and software have made it possible to use a generalized linear models (GLMM) approach using a Poisson or negative binomial error structure. In doing so, it is possible to accommodate overdispersion in a direct manner. This approach has intuitive appeal and has recently attracted much interest in ecotoxicology. Both NOEC and regression approaches to data interpretation are considered. For the NOEC approach, the power of various tests to find effects will be explored through extensive computer simulations and through a large database of guideline studies. Results from GLMM models with Poisson error structure will be compared to the previous standard approach of approximating a normal error structure on (possibly transformed) replicate counts and applying Williams' or Dunnett's test or applying a non-parametric alternative using the stepdown Jonckheere-Terpstra or Shirley test or a Dunn or Mann-Whitney pairwise analysis. For the regression approach, a comparison will be made of the quality of ECx estimates, in terms of precision, bias, uncertainty, and goodness of fit, from GLMMs with Poisson error structure and gamma priors to accommodate overdispersion, to familiar non-linear models based on an approximate normal distribution of (possibly transformed) within-treatment replicate proportions. An application will be made to the recently issued mollusc reproduction test guideline. The choice of analysis has important risk assessment implications and it is important to understand the strengths and weaknesses of various approaches in order to make scientifically sound and practical choices.

RP236 Comparison of Statistical Approaches for Quantal Data from Ecotoxicity Studies

J.W. Green, DuPont / Applied Statistics Group

Quantal data is a type of count data that often arises in ecotoxicity study. A fixed number of subjects are exposed and the interest in is the number of those subjects that respond, e.g., die or develop a specific type of lesion or condition. There numerous ways to analyze quantal data. For NOEC determination, these include the Cochran-Armitage test, with a Rao-Scott adjustment in case of overdispersion, Williams' or Dunnett's test on transformed data to approximate normality. Recently, there has been much interest in applying generalized linear mixed models (GLMM) using a binary error structure (that accommodates overdispersion where needed) to quantal data. Williams' beta-binomial tests is an older approach that is a specific way to implement GLMM. GLMM models have good conceptual appeal and provide natural extensions to regression from which a percent effects level (ECx) can be estimated. This paper will explore through extensive computer simulation modeling and a substantial database of guideline studies the relative merits of these alternative approaches in terms of their power and sensitivity to find effects. For the regression approach, a comparison will be made of the quality of ECx estimates, in terms of precision, bias, uncertainty, and goodness of fit, from probit or logistic models based on binary distributions, familiar non-linear models based on an approximate normal distribution of (possibly transformed) within-treatment replicate proportions, and the same non-linear models for the mean response but using GLMMs with binary error structure that accommodate overdispersion. The choice of analysis has important risk assessment implications and it is important to understand the strengths and weaknesses of various approaches in order to make scientifically sound and practical choices.

RP237 Issues for ECp Estimation

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For chronic toxicity to aquatic organisms, the past 30 years have seen a growing interest in the estimation of concentrations causing a certain percentage effect (ECp) rather than using highest no-observed effect concentrations (HNOEC) or lowest observed effect concentrations (LOEC) as the basis for risk characterizations. Although ECp estimation has certain advantages for better defining risk, it involves various issues regarding estimation methodology, model selection, and data sufficiency. Using a "mega" dataset regarding the chronic toxicity of NaCl to Ceriodaphnia dubia, various issues and potential problems in ECp estimation for more typical data sets will be explored.

RP238 Bayesian modeling of the co-occurrence of aquatic species and contaminants: Model evaluation and case study application

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Risk managers would greatly benefit from modeling tools that facilitate probabilistic assessment of potential ecological exposures in natural ecosystems at the landscape or watershed scale. For aquatic systems, empirical data on contaminants (e.g., metals, pesticides, invasive species) and species distributions (e.g., fishes) are often readily available from sources such as agency bioassessment programs. Such data could be utilized to model and map exposure risk in terms of the probability of co-occurrence of target species and contaminants across the landscape. For this paper, we evaluated a Bayesian model utilizing Hamiltonian Monte Carlo (HMC) for application of the multivariate probit (MVP) model to the problem of modeling the joint occurrence of contaminants and species distributions using empirical data. As an alternative to developing individual models (e.g., logistic regression) for each response (i.e., chemical occurrence and fish occurrence) and multiplying the probabilities to arrive at a conditional probability of co-occurrence, the MVP model estimates the joint distributions for multiple responses in a single, multivariate model; accounting for the potential dependence between empirical contaminant and species occurrences. We first evaluated the model using simulated data and then applied it to a case study watershed, where we predicted and mapped the probability of co-occurrence of agricultural pesticides and sensitive stream fishes.

RP239 Genotoxic response assessment in the Neotropical fish by alkaline unwinding assay using methyl methane sulfonate (MMS) as chemical model

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The aims of this study was to assess the genotoxic response, single-strand (SSB) and double-strand breaks (DBS), in Cnesterodon decemmaculatus by a fluorometric microscale alkaline unwinding assay using MMS as reference compound. Assay was first set up in vitro generating single strand-breaks by enzymatic digestion with calf thymus endonuclease I on DNA samples obtained from control fish skeletal muscle. Then, in vivo assays were performed exposing fish to 0, 3.75, 7.5, and 15 mg L^{-1} MMS to build the dose-response curve. Sybr Green I was used as fluorochrome and DNA unwinding was performed on a multi-mode microplate reader. The strand scission factor (SSF) was assessed as endpoint in both assays. Good correlation between digestion time and SSF was observed from 0 to 30 min and 0.65 to 2.17 arbitrary DNA damage units. Clear genotoxic induction was also observed in fish muscle during in vivo assays. The SSF value was increased from 0.15 in controls to 0.45, 0.50 and 0.54 in 3.75, 7.5, and 15 mg L^{-1} MMS expose fish, respectively. Significant effects were observed earlier from 3.75 mg L^{-1} (LOEC), and the EC_{50} was estimated between 0 and that value. In conclusion the developed fluorometric microscale alkaline unwinding assay was sensitive to assess genotoxic effects induced by the reference chemical in the tested South American fish. Therefore, it appears as an easy, quick and reliable tool for assessing genotoxicity in the lab, and potentially in the field using a local species.

RP240 Drug resistance-related major vault protein (MVP) and cellular vault particles in fish: A piece of the puzzle in understanding multi-drug resistance?

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Cellular vaults are ubiquitous 13 mega Da multi-subunit organelles that may have a role in nucleo-cytoplasmic transport. Seventy % of the vault's mass consists of a ≈100 kDa protein, the major vault protein (MVP). Earlier work in our lab sequenced MVP from channel catfish, Ictalurus punctatus, monocytes (42TA cells). We recently expressed channel catfish MVP as a recombinant protein for generating a panel of specific mAbs, resulting in hybridoma 3F9 secreting a mAb that recognizes MVP in all fish examined to date, as well as mammalian cells. Using immunohistochemistry, we show that mAb 3F9 staining for MVP (and presumably intact vaults) is highly expressed in epithelial, immune system, and endothelial cells. Using qPCR, MVP mRNA expression is induced in channel catfish ovarian cells by ethidium bromide, which damages DNA. We also examined MVP protein expression in tissues from Atlantic killifish collected in situ at the Atlantic Wood (AW) EPA superfund site. Killifish from the AW site display a PAH-resistant phenotype, and had a high incidence of liver lesions and neoplasia. Through immunoblotting and IHC, we show that MVP protein expression is highly variable in AW fish compared to fish from a reference site. Also, MVP is highly expressed in many advanced neoplastic liver lesions, with nuclear location in the most advanced lesions. The true function of cellular vaults remains unknown, but the results of this study support a possible role in acquired resistance to intracellular toxic compounds, and or their metabolites.

RP241 Aquatic insects as a mechanism of dispersal for antibiotic resistance genes in the environment

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Human-use antibiotics are commonly released into aquatic ecosystems from wastewater effluents emanating from lagoons on the Canadian Prairies. This can lead to selection for antibiotic resistant gene-bearing bacteria (ARGs) in these environments (both lagoons and receiving waters). We hypothesize that as aquatic insects emerge from these lagoons, they could be a vector for ARGs into surrounding environments. To test this, we deployed emergence traps in three wastewater lagoons and the effluent-receiving creek at Dunnottar, MB to determine the type and abundance of aquatic insects emerging from wastewater lagoons. The ARGs were measured on emerged insects, in the water and were quantified using qPCR. The antibiotic resistance genes targeted were sul1, sul2, and sul3 and were compared to 16S-rRNA to calculate the proportion of resistant genes relative to total bacterial genes. The concentrations of sulfonamide antibiotics were characterized as well. The greatest proportion of sulfonamide resistant genes relative to 16S-rRNA was 9.3% and was measured in the primary lagoon, with lesser amounts observed in the rest of the wastewater system. We were not able to detect sul-type resistant genes within emerging insects, suggesting that this mechanism of spread is unlikely to be significant compared to other means (e.g., direct release of improperly treated wastewaters).

RP242 Assessment of biochemistry and genotoxicity in Rhamdia quelen feed with residual algae biomass from biodiesel production

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Climate change caused by the release of greenhouse gases by burning of fossil fuels has created an appeal for sustainable energy production. The difficulty of obtaining renewable fuels is the application of energy-compensating systems to meet the demand. The cultivation of microalgae is an alternative for the production of biodiesel. Microalgae can be created in different culture media, including industrial effluents. After processing of microalgae, a residue called defatted biomass waste (RBD) is obtained, and it can be used as an ingredient in animal feed production. The microalgae Acutodesmus obliquus (Green algae) (Scenedesmus obliquus) was cultivated in medium (Chu), and it was used in the production of feed. 60 fingerlings of the species Rhamdia quelen (Catfish) were used. Four groups (n=15) were created: control (normal feed – 0%) and other 3 groups with feed enriched with RDB in the proportion of 1%, 2% and 3%. The feed lasted 60 days (the fish were fed three times daily ad libitum). The biomarkers were comet assay (for erythrocytes, hepatocytes and brain cell analyses), piscine micronucleus and halo assay (for erythrocytes), acetylcholinesterase (AChE) for the brain and muscle analyses. For liver cells, the biochemical biomarker used were catalase (CAT), glutathione S-transferase (GST), superoxide dismutase (SOD) and lipid peroxidation (LPO). Normal data were analyzed using one-way variance (ANOVA). Non-normal data were analyzed using Kruskal-Wallis. In the comet assay, it was not observed brain tissue damage. A significant decrease in DNA damage in erythrocytes from concentrations 2% and 3% was observed, as well as in concentrations 3% RBD liver tissue. No difference was observed between the groups in the piscine micronucleus. There was an increase in AChE enzyme activity in the brain for the 3% group, and there was an increase of muscle activity in groups with 2% and 3% RBD. The degradation of intracellular hydrogen peroxide (CAT) was not changed. The GST enzyme also showed no change. SOD metaloenzyme, which catalyzes the dismutation of the superoxide, showed an increase in activity for groups with 2% and 3% of RBD. The measuring of the LPO showed no loss of cell membrane integrity. Microalgae are major producers of antioxidants and a food containing these antioxidants are known to be beneficial. The evaluation of safety in the use of RBD in fish feed is important to prevent future risks to the health of its consumers.

RP243 The Effect of Starvation and Refeeding on Global Gene Expression in Livers of Rainbow Trout (Oncorhynchus mykiss)

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During their life cycle, salmonids may face extended periods without food. Fish depend on body energy reserves during these periods of starvation. An effective way to identify the relationships between major metabolic pathways and body processes is to examine changes in metabolism during starvation followed by a refeeding. The present study investigated the global gene expression following a 14 day starvation period and a 14 day refeeding period in rainbow trout. While no significant effects were seen in length and body weight following the 14 day starvation period, hepatosomatic index (HSI) was significantly reduced. Length and body weight of rainbow trout were significantly increased following the 14 day refeeding period while HSI increased to pre-starvation levels. RNA-seq analysis was utilized to determine the differentially expressed genes in liver extracts. Results from the present study will provide possible mechanisms of metabolic adaptation of liver tissues to starvation and refeeding of rainbow trout. In addition, the results may provide an indication into whether starvation can lead to enhanced toxicity of environmental contaminants.

RP244 Use of siRNA to Understand the Role of Hemoglobin in the Toxicity of Nitrite

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Hemoglobin levels in some aquatic species vary with oxygen availability. Environmental nitrogenous compounds, such as nitrate and nitrite, can bind to the heme moiety of hemoglobin resulting in toxicity. We hypothesized elevated hemoglobin levels resulting from low oxygen conditions can increase the tolerance of daphnids (Daphnia magna) to nitrite by sequestering the nitrite yet providing ample unaltered hemoglobin to meet the oxygen transport needs of the organisms. We investigated this by: 1) comparing the toxicity of nitrite to control daphnids and daphnids with chemically (pyriproxyfen)-induced hemoglobin levels; and, 2) evaluating the restoration of sensitivity to nitrite in pyriproxyfen-treated daphnids by suppressing translation of the hemoglobin hb1 and hb2 genes using small interfering RNA (siRNA). Pyriproxyfen exposure increased hemoglobin levels as evidenced by increased hb1 and hb2 mRNA levels and increased red coloration of the organisms. Pyriproxyfen exposure also increased the tolerance of daphnids to the acute toxicity of nitrite. Feeding daphnids siRNA that specifically targeted hb1 and hb2 mRNA significantly attenuated the induction of hemoglobin by pyriproxyfen and the tolerance of these organisms to nitrite toxicity. The suppression of hemoglobin expression was monitored throughout the experiments by noting the reduced red coloration of the daphnids and was confirmed at the end of the experiments by quantitative real-time PCR. Results support the hypothesis that the induction of hemoglobin serves to protect daphnids from the toxicity of some environmental chemicals. Further, results of this study demonstrate that feeding of siRNA is an efficient, non-invasive means to knock down specific gene products in daphnids.

RP245 Identification and evaluation of reference genes for the normalization of qRT-PCR analysis in Crassostrea brasiliana

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Accurate quantification of transcripts using quantitative real-time PCR (qRT-PCR) depends on the identification of reliable reference genes for normalization. Despite the lack of studies on reference genes selection in bivalves, gene transcription studies using real-time quantitative reverse transcription PCR (qRT-PCR) have notably increased in these organisms. For oysters, a systematic evaluation of reference genes under environmental stressors and contaminant challenges is required. The present study aimed to contribute to the identification of the most suitable reference genes for normalization of mRNA transcription data in qRT-PCR analysis of Crassostrea brasiliana. For this purpose, mRNA expression of nine candidate genes were analyzed in the gills of oysters under three different experimental conditions: (i) different temperatures (18°C, 24°C or 32°C) and phenanthrene (100 $\mu g.L^{-1}$) combined exposure; (ii) different salinities (10, 25 or 35) and phenanthrene (100 $\mu g.L^{-1}$) combined exposure and (iii) 10% of diesel fuel water-accommodated fraction (diesel WAF) exposure. Reference gene transcription stability was calculated using four algorithms (geNorm, NormFinder, BestKeeper, comparative delta Ct), and the recommended comprehensive ranking was provided using RefFinder tool. The geometric means of ranking values using the RefFinder tool identified several optimal sets of genes that are suitable for qRT-PCR data normalization: transcripts of ankyrin-like, GAPDH-like and α tubulin-like genes had the three lowest variations in temperature and PHE combined exposures; transcripts of ankyrin-like, β actin-like and β tubulin-like genes were optimal for salinity and PHE combined exposure; and transcripts of α tubulin-like, 28S ribosomal protein-like and ankyrin-like genes were the most stable reference genes for diesel WAF exposure. Throughout all experimental conditions, the genes with the lowest global variability were ankyrin-like and α tubulin-like across all durations of exposure. These results revealed that the reference genes ankyrin-like and α tubulin-like reported here could be employed for differential expression in future studies using qRT-PCR with similar exposure conditions in C. brasiliana. Supported by: CNPQ/INCT-TA, CAPES/ CNPq

RP246 Tissue-based mapping of the fathead minnow (Pimephales promelas) transcriptome and proteome

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The fathead minnow (FHM, Pimephales promelas) is used extensively to explore effects of contaminant exposure in both field and laboratory settings due to its regulatory importance, ease of culture, and the existence of extensive species-specific biology and toxicity data. The availability of genetic information and molecular tools specific to the FHM pales in comparison to that of the zebrafish (Danio rerio) despite its limited ecological relevance and regulatory importance. As such, there has been increased interest to bring the FHM into the genomic era to increase our understanding of complex mechanisms of action of environmental contaminants through the use of functional genomic applications, particularly in the development of adverse outcome pathways. To address this gap in genetic information, we sought to build a FHM transcriptomic and proteomic sequence database. For the transcriptome sequence database construction, RNA was collected from gut, liver, gonad, brain, blood, heart, and kidney from one healthy adult male fish, a cDNA library was constructed and then sequencing was performed on a PacBio instrument. Transcripts were mapped using publicly available zebrafish and human databases resulting in over 70,000 annotated genes. Protein sequences were generated and the largest open reading frame for each gene was selected and annotated using Blast2Go, resulting in 65,000 mapped protein sequences. To create transcriptomic and proteomic maps, we collected RNA and protein, respectively, from gut, liver, telencephalon, and hypothalamus tissues. Global RNA expression was measured by RNAseq and global protein expression was measured by iTRAQ labeling followed by LC-MS/MS analysis. Transcripts and proteins were mapped using the sequence databases generated in house and their relative expression levels in each tissue were compared. Results revealed differential expression of transcripts and proteins across tissues that in some cases were not consistent at the gene and protein levels. These results highlight the need for comprehensive transcriptomic and proteomic analyses of contaminant effects as changes in expression may not be conserved across all levels of regulation. These results also provide the first tissue-based transcriptomic and proteomic maps for FHMs that will have importance in functional genomic applications in ecotoxicology.

RP247 The role of mitochondrial dysfunction in the adverse outcome pathway for impaired growth

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Several studies suggest that many chemicals in the environment alter mitochondrial function. Changes in mitochondrial function, such as those related to basal metabolism and ATP production, may affect ecologically relevant endpoints such as growth. Although there are robust models to assess mitochondrial function for human health, there remains a critical research need to address these effects in ecologically-relevant species like the fathead minnow, which is found throughout North America. Thus, the objectives of this study were to: (1) Develop an assay to measure mitochondrial function in fathead minnow embryos and (2) Link mitochondrial function and growth in an adverse outcome pathway. Here, the Seahorse XFe24 Extracellular Flux Bioanalyzer was used to measure oxygen consumption rate (OCR) in embryos at various life stages. Our optimization studies have determined that one embryo per well is sufficient to detect an OCR of 200 (24 hr stage) to 250 (48 hr stage) pmol/min, which is within the dynamic range of the instrument (50-400 pmol/min). Similarly, basal respiration and ATP production increased with age. Next, a known mitochondrial toxicant, 2,4-dinitrophenol, was used to assess changes in mitochondrial function and growth. Fathead minnow embryos were exposed at three doses (1, 10, and 100 μg/L) for 24 hours and assayed for changes in basal respiration, ATP production, maximal respiration, and spare capacity. Fathead minnows were also exposed to these conditions for 7 days and measured for total length using ImageJ. Basal and maximal respiration decreased for all doses of 2,4-dinitrophenol and demonstrated a dose-dependent relationship. Similarly, there was also a decrease in growth at higher doses of 2,4-dinitrophenol after 7 days of exposure. Thus, results indicate an association between mitochondrial dysfunction and growth, which may be used to develop an adverse outcome pathway for this important ecological endpoint.

RP248 A new genomic resource for the fathead minnow (Pimephales promelas): Development and application of a genetic linkage map

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The fathead minnow (Pimephales promelas) is the most widely-used small fish model for regulatory ecotoxicology in North America. However, the genome-scale resources and molecular genetic tools needed to exploit the full potential of this aquatic toxicological model are lacking. Specifically, the absence of structural genome information for the fathead minnow currently limits its use in the dissection of complex traits, genetic marker discovery, identification of gene regulatory domains, and elucidation of directed biological networks that underlie response to chemical stress. To overcome this deficit, we developed a novel, SNP based consensus genetic linkage map based on three independent genetic crosses. The resulting genetic architecture defines 25 major linkage groups, consistent with the number of chromosomes in the fathead genome. To illustrate the utility of this newly developed tool, we performed a quantitative trait loci (QTL) analysis of the phenotype SEX. Multiple QTL were identified, which together, explain the majority of genetic variance (77%, p = 6.34 x 10⁻¹²) observed for fathead gender. These genetic markers may have utility for determining the genetic sex in intersex individuals by distinguishing feminized males from masculinized females. This will be of paramount interest to ecotoxicologists using the fathead minnow as a model to investigate sensitivity to endocrine disruption and sex-reversal.

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