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Advancing human health risk assessment

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Abstract

The current/traditional human health risk assessment paradigm is challenged by recent scientific and technical advances, and ethical demands. The current approach is considered too resource intensive, is not always reliable, can raise issues of reproducibility, is mostly animal based and does not necessarily provide an understanding of the underlying mechanisms of toxicity. From an ethical and scientific viewpoint, a paradigm shift is required to deliver testing strategies that enable reliable, animal-free hazard and risk assessments, which are based on a mechanistic understanding of chemical toxicity and make use of exposure science and epidemiological data. This shift will require a new philosophy, new data, multidisciplinary expertise and more flexible regulations. Re-engineering of available data is also deemed necessary as data should be accessible, readable, interpretable and usable. Dedicated training to build the capacity in terms of expertise is necessary, together with practical resources allocated to education. The dialogue between risk assessors, risk managers, academia and stakeholders should be promoted further to understand scientific and societal needs. Genuine interest in taking risk assessment forward should drive the change and should be supported by flexible funding. This publication builds upon presentations made and discussions held during the break-out session ‘Advancing risk assessment science – Human health’ at EFSA’s third Scientific Conference ‘Science, Food and Society’ (Parma, Italy, 18–21 September 2018).

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Table of contents

Abstract................................................................................................................................................... 1
1. Introduction........................................................................................................................................ 4
  1.1. Advancing human health risk assessment – concepts ................................................................. 4
  1.1.1. The NASEM reports envisioning the future of risk assessment: opportunities and challenges.... 4
  1.2. Holistic human health risk assessment: challenges to a fit-for-purpose approach .................... 5
  1.3. New approach methods in toxicology for mechanism-based hazard assessment .................... 5
  1.4. Use of epidemiological studies for chemical risk assessment: strengths and limitations .......... 6
  1.5. Risk communication and how simple heuristics influence laypeople’s risk perception ............ 6
2. Advancing human health risk assessment – new tools, new approaches in exposure assessment and examples of their integration ......................................................................................................... 7
  2.1. New tools .................................................................................................................................... 7
  2.1.1. NAMs for new chemical safety testing strategies: the EU-ToxRisk project.............................. 7
  2.1.2. Assessment of chemical mixture-induced developmental neurotoxicity using human in vitro model..... 7
  2.1.3. The use of toxicogenomics in chemical risk assessment.......................................................... 8
  2.1.4. Integrating pharmacokinetics and pharmacodynamics in AOPs for next generation risk assessments.. 8
  2.1.5. Modelling tools to assess risks related to cadmium exposure for workers and consumers.......... 9
  2.1.6. Predictive tools in the risk assessment of new proteins in genetically modified organisms (GMOs): the case of celiac disease ................................................................. 10
  2.2. New approaches in exposure assessment ....................................................................................... 10
  2.2.1. Human biomonitoring ................................................................................................................ 10
  2.3. Holistic assessment of exposures to environmental and endogenous oestrogens by internal dosimetrics ................................................................. 11
  2.4. The exposome in practice ............................................................................................................ 12
  2.5. Integrating new tools and new approaches in exposure assessment – examples ......................... 13
    2.5.1. Integrated safety assessment of genetically modified food/feed: the experience from the EU projects GRACE and G-TwYST ................................................................. 13
    2.5.2. CLARITY-BPA Project: Core NCTR/NTP study on BPA and lesson learnt on integrating regulatory and academic investigations in hazard assessments by the US National Toxicology Program ................................................... 14
    2.5.3. Setting a health-based guidance value using epidemiological studies ...................................... 15
3. Conclusions and recommendations ................................................................................................ 15
References ............................................................................................................................................... 16
Abbreviations ........................................................................................................................................... 20
1. **Introduction**

The current human health risk assessment is substantially hazard driven and based on world-wide recognised protocols largely relying on animal studies. Translatability of results from these studies to humans and considerations on the replacement, reduction and refinement of animal studies are a matter of debate. In parallel, biological and toxicological sciences today can benefit from paramount scientific and technological advances. In relation to hazard assessment, new tools are emerging that enable a better understanding of the mechanisms leading to adverse effects, more accurate predictions of biological responses and so help to establish causality, offering the benefit of being, in most cases, non-animal test models. Exposure science is also rapidly developing and epidemiological research is facing a transition from empirical observations alone to a molecular epidemiology paradigm incorporating exposure and pathogenesis. All these developments are promising and support a shift from the current risk assessment paradigm to a more holistic approach, improving assessment of human health risk while reducing animal testing.

A driver for this shift is the National Academies of Sciences, Engineering and Medicine (NASEM) that, since 2007, has published reports providing a vision and a proposal for strategy for the toxicology of the 21st century based on new tools and approaches, developing the exposure science and proposing the integration of these tools to further advance risk assessments. These reports served as the basis for various initiatives in the United States and world-wide accelerating data generation and, in general, the effort for such a paradigm shift. Elaboration on the need/trend of a new conceptual framework in human health risk assessment, supported by new concepts and tools in hazard assessment and exposure has been proposed in the European Union (EU) in a SCHER/SCENIHR/SCCS report in 2013.\(^1\)

However, it is recognised that the implementation of new approaches and tools and the use of new data generated face challenges impacting the paradigm shift, as well as acceptance by regulators. Transparent and understandable communication of the strengths and weaknesses of new approaches is pivotal to their application and acceptance by the scientific community, regulators and laypeople.

This publication is intended to present an overview of the general concepts at the basis of a possible shift from traditional to holistic risk assessment and to apply the available relevant scientific methodologies and technological advances that may entail such a shift, together with challenges and needs, including:

- opportunities and challenges related to the application of new tools and technologies for the identification and characterisation of adverse effects;
- the role of human epidemiology in the identification and characterisation of health effects induced by chemicals;
- the integration of human biomarkers in exposure assessment.

While focusing on chemicals, many of the considerations discussed would be applicable to other stressors.

Concrete case studies utilising new tools, new approaches for exposure assessment and their integration are also presented.

This publication builds upon presentations made and discussions held during the break-out session ‘Advancing Risk Assessment Science – Human Health’ at EFSA’s third Scientific Conference ‘Science, Food and Society’ (Parma, Italy, 18–21 September 2018). Additional discussions relevant to the topic are presented in this issue by Cavalli et al. (2019), Hartung (2019) and Hougaard Bennekou (2019).

1.1. **Advancing human health risk assessment – concepts**

1.1.1. **The NASEM reports envisioning the future of risk assessment: opportunities and challenges**

In 2007, the NASEM released the report ‘Toxicity testing in the 21st century: a vision and a strategy’ (NRC, 2007), which capitalised on the advances in biology and related fields and increases in computational power to envision a future in which toxicity testing primarily relies on high-throughput *in vitro* assays and computational tools to assess potential adverse effects from chemical exposure. A

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vision for exposure science was articulated several years later in the National Academies report, ‘Exposure science in the 21st century: a vision and a strategy’, (NRC, 2012), which expanded the breadth and depth of exposure science given advances in, for example, monitoring technologies, analytical techniques and computational tools. Since the release of those reports, various agencies and organisations have started to collaborate within and outside the United States to advance the visions. Generation of diverse data streams from government, industry and academic laboratories has accelerated. Although scientists and others expect that implementation of the visions will take decades to be fully achieved, the recent National Academies report, ‘Using 21st century science to improve risk-related evaluations’ (National Academies of Sciences, Engineering, and Medicine, 2017), examines how the data being generated today can be used in risk assessment applications. Four areas (priority setting, chemical assessment, site-specific assessment and assessment of new chemicals) have been identified that could benefit from incorporating the 21st century science, and case studies have been described. Although there are many technical issues still to be resolved, the report identified one particular challenge that looms large. Technology has evolved far faster than our ability to analyse, interpret and integrate the diverse, complex and large data streams for risk assessment. The path forward to address the challenges entails a research agenda that develops, explores and documents case studies capturing various scenarios of data availability for risk assessment applications. Multidisciplinary collaboration will also be critical. Ultimately, application and acceptance of the new approaches will depend on communicating the strengths and weaknesses in a transparent and understandable way.

1.2. Holistic human health risk assessment: challenges to a fit-for-purpose approach

There is consensus that the 21st century paradigm shift in human health risk assessment will be based on the understanding of mechanisms of toxicity rather than on the identification of apical endpoints of toxicity. This mechanistic shift has great potential for improving human health risk assessment and tailoring it to different problem formulations. The transition to a mechanism-based risk assessment of chemicals would require a holistic approach in which several aspects should be considered: the identification and use of adverse outcome pathway (AOP) as a framework that integrates new approach methods (NAMs) supporting mechanistic understanding and predictive screening; electronic data availability; rigorous analysis of uncertainties; definition of protection goals; and flexible and where necessary tailored data requirements, and harmonised approaches. A key question is whether current EU regulations are flexible enough to take full advantage of this potential. In this context, several aspects should be considered:

- Are the standard requirements for risk assessment of human health fit for purpose?
- Is there the need for more flexibility in requirements and in the accompanying guidance documents?
- Are existing data used at their best?
- Can mutual recognition of the risk assessment outputs be recognised?

This discussion is further developed in this issue by Hougaard Bennekou (2019).

1.3. New approach methods in toxicology for mechanism-based hazard assessment

Many animal-based test methods have never been formally validated; their predictivity for complex endpoints, such as cancer or developmental toxicity, is sometimes poor (60–70% range) and may for specific cases (e.g. murine liver tumours) be questionable altogether. Moreover, interspecies extrapolations are a large challenge. Animal-based testing, e.g. for developmental neurotoxicity (DNT), is also extremely demanding in terms of resources. Finally, this approach has a low throughput and yields little or no information on the mechanism of toxicity (Hartung and Leist, 2008; Leist and Hartung, 2013; Daneshian et al., 2015; Meigs et al., 2018).

An alternative approach to animal testing, as suggested by the NASEM (Leist et al., 2008), and large groups of scientists world-wide (Basketter et al., 2012; Ramirez et al., 2013; Leist et al., 2014, 2017; van Vliet et al., 2014; Gordon et al., 2015; Rovida et al., 2015; Marx et al., 2016) is the use of combinations of in vitro (e.g. cell cultures, organoids, zebra fish embryos) and in silico (e.g. QSAR, PBTK) methods. To distinguish these novel approaches for identification and quantification of chemical
hazard from traditional animal experiments, they have been called NAM. Notably, in some fields, the term NAM is used with a more extended scope to also include new types of animal studies, or to comprise the use of pre-existing animal data for known compounds in a read-across procedure to predict safety/hazard of new compounds. However, the term is used in this paper to imply only animal-free new approaches as pursued by the H2020 research project EU-ToxRisk (Daneshian et al., 2016). To use NAMs in a regulatory context, a process and criteria to set out their readiness are essential. The readiness evaluation extends the scope of classical method validation by allowing different (fit-for-purpose) readiness levels for various applications. The field of DNT testing can serve to exemplify the application of readiness criteria. The number of chemicals not tested for DNT, and the testing cost per chemical are overwhelming for in vivo DNT testing. So, there is a need for inexpensive, high-throughput NAM approaches, to obtain initial information on potential hazards, and to allow prioritisation for further testing (Bal-Price et al., 2015, 2018; Aschner et al., 2017; Fritsche et al., 2017, 2018). Based on readiness criteria, a (semi)-quantitative analysis process was assembled on test readiness of 17 NAMs with respect to various uses (e.g. prioritisation/screening, risk assessment). The scoring results suggest that several assays are currently at high readiness levels. Therefore, DNT NAMs may be assembled into an integrated approach to testing and assessment (IATA). Furthermore, NAM development may be guided by knowledge of signalling pathways necessary for normal brain development, DNT pathophysiology and relevant AOPs. There is, however, an educational need on all sides to understand strengths and weaknesses of the new approaches (Kadereit et al., 2012; van Thriel et al., 2012; Smirnova et al., 2014; Schmidt et al., 2016; Hartung et al., 2019).

1.4. Use of epidemiological studies for chemical risk assessment: strengths and limitations

Chemical risk assessment should ideally be based on studies carried out under controlled experimental conditions. However, for humans, such experiments cannot be performed for potentially harmful substances. Although human observational studies can be used as an alternative, the time needed to generate reliable results for new substances and methodological limitations have traditionally hampered their use. For these reasons, procedures and regulatory framework for chemical risk assessment have largely been driven by reliance on experimental studies in animals. The limitations of that approach are uncertainties related to extrapolating findings from animals to humans and use of doses that are usually far higher than those observed in humans. Although these limitations can be partly reduced by use of safety factors, it is increasingly acknowledged that precision in risk assessment can be improved by incorporating findings from human observational studies. Recent advancements in analytical chemistry in terms of rapid method development, reduction in sample volume and cost, as well as improved access to computerised health data have made human observational studies of sufficient quality more frequently available for risk assessors. Apart from general limitations in terms of bias (including confounding), use of human studies compared with animal studies is more complicated due to occurrence of other co-exposures and the fact that unexposed individuals usually do not exist. The quality of the exposure assessment is crucial. The high variability in term of susceptibility to chemical exposures and interaction with other lifestyle factors means that results from different human studies can be conflicting. The current framework for chemical risk assessment is generally not compatible with these complexities and compromises are needed. A better understanding of strengths and weaknesses of traditional toxicological studies and human observational studies is the key for further advancing the methodology for chemical risk assessment.

1.5. Risk communication and how simple heuristics influence laypeople’s risk perception

Among aspects to be considered facing this change in risk assessment paradigm, of utmost relevance is the risk communication versus laypeople. The qualitative characteristics of a hazard, and not relevant quantitative information, strongly influence laypeople’s risk perception. Recent research (Siegrist and Sütterlin, 2017; Scott et al., 2018) has focused on the role of simple heuristics: natural is good and synthetic is bad, for example, in people's hazard evaluations. The results of such studies indicate that not only the negative consequences of a hazard, but also whether the hazard is human-made (e.g. glyphosate) or naturally occurring (e.g. Campylobacter) has a significant influence on people's perceptions. Indeed, negative outcomes are perceived to be more severe if they are
anthropogenic than if they stem from nature. Perceiving gene technology to be unnatural also seems to be a significant reason why the risks as well as the benefits associated with this technology are perceived differently when compared with the risks and benefits associated with conventional breeding technology. Another heuristic that people may apply when evaluating the healthiness of foods is that the absence of certain substances implies the product is healthier. Therefore, products with ‘free from’ labels (e.g. free from palm oil, free from genetically modified (GM) organisms) are perceived to be healthier than products without such labels. Biased decisions that result from people’s reliance on simple heuristics have been observed in different contexts, and they may result in non-optimal decisions being made. However, providing information to laypeople seems to have only a limited impact on how hazards are perceived. This poses a challenge for risk communication intended to change laypeople’s perceptions so that they will fall more in line with the best available scientific evidence.

2. Advancing human health risk assessment – new tools, new approaches in exposure assessment and examples of their integration

2.1. New tools

2.1.1. NAMs for new chemical safety testing strategies: the EU-ToxRisk project

The large-scale EU-ToxRisk project (http://www.eu-toxrisk.eu/) is an integrated European ‘flagship’ programme with the vision to establish a paradigm shift in toxicity testing and risk assessment for the 21st century by implementing mechanism-based integrated testing strategies using non-animal NAMs. To accomplish this, the EU-ToxRisk project has united all relevant scientific disciplines covering in silico QSAR modelling, cellular toxicology, bioinformatics and physiologically based pharmacokinetic (PBPK) modelling. The project tests the integration of the different NAMs in various case studies, ultimately establishing: (i) pragmatic, read-across procedures incorporating mechanistic and toxicokinetic (TK) knowledge; and (ii) ab initio hazard and risk assessment strategies for chemicals with little background information. The case studies are focused on repeated dose systemic toxicity (RDT) targeting either liver, kidney, lung or nervous system toxicity, as well as developmental/reproduction toxicity. The integration of the various NAMs in defined case studies allows the assessment of the overall applicability domain of these NAMs in chemical hazard and ultimate risk assessment. Case studies are centred around AOPs and include, for example, the application of NAMs for the assessment of: (i) microvesicular liver steatosis induced by valproic acid analogues; (ii) the prediction of teratogenic effects of valproic acid analogues; and (iii) the application of NAMs to assess the AOP pathway related to inhibition of the mitochondrial respiratory chain complex 1 of nigra striatal neurons leading to parkinsonian motor deficits. Importantly, the activities in the case studies are supported and guided by both cosmetics, (agro)-chemical, pharma industry stakeholders as well as various European regulatory authorities. The final goal is to deliver testing strategies to enable reliable, animal-free hazard and risk assessment of chemicals based on a mechanistic understanding of chemical toxicity.

2.1.2. Assessment of chemical mixture-induced developmental neurotoxicity using human in vitro model

Chemicals that are known to trigger-specific DNT effects belong to different chemical classes including industrial chemicals, persistent organic pollutants (POPs), metals and pesticides. They belong to multiple regulatory silos related to food and food quality such as pesticides, food contact materials and food additives, including flavourings, colourings and preservatives. These examples illustrate that common, similar or related toxic effects triggered by various chemicals may be differently regulated and that the combined effects of these chemicals across different regulatory domains are not currently considered. At the same time, it is well documented in the existing literature that ‘mixture effects’ can be greater than effects triggered by the most potent single chemical in a mixture, and the mixture effects may be additive, or in some cases even synergistic. Therefore, implementation of mixture risk assessment (MRA) for DNT evaluation, is strongly advocated as infants and children are indisputably co-exposed to more than one chemical at the time. Indeed, for example, breast milk has been found to contain chemicals regulated as pesticides, along with those regulated as cosmetics (including UV filters parabens, phthalates), together with POPs including polychlorinated biphenyls (PCBs), confirming
that simultaneous co-exposure to multiple chemicals occurs in babies and during pregnancy (Schlumpf et al., 2010; de Cock et al., 2014). A challenge in evaluation of DNT effects induced by chemicals is that the neurodevelopmental outcome depends not only on the kind of exposure (dose, duration) but also on the developmental stage of the brain at the time of exposure.

Therefore, in this study, it was proposed to use a mixed culture of neuronal and glial cells derived from human induced pluripotent stem cells as this in vitro model makes it possible to evaluate a chemical impact on key neurodevelopmental processes (including cell proliferation, migration and morphological-functional neuronal and glial differentiation) mimicking critical stages of human brain development. Moreover, the applied in vitro assays were anchored to the selected neurodevelopmental processes that overlapped with common key events identified in AOPs relevant to impairment of learning and memory in children; this is the most frequent adverse outcome identified in the existing DNT AOPs (Bal-Price and Meek, 2017). The effects of the selected compounds (administered as a single chemical or in mixtures) were assessed on human neural precursor cells undergoing differentiation to determine synergistic, antagonistic or additive effects on brain-derived neurotrophic factor (BDNF) level, neurite outgrowth and synaptogenesis after short- (72 h) or long-term exposure (14 days in vitro). The obtained data suggest that low, non-/cytotoxic concentrations, below lowest-observed-effect concentrations (LOAECs) of single chemicals become neurotoxic in mixture, especially for the chemicals working through a similar mode of action (MOA) and after 14 days of exposure.

2.1.3. The use of toxicogenomics in chemical risk assessment

‘Omics methods addressing the whole genome (genomics), the transcriptome (transcriptomics), the proteome (proteomics) and the metabolome (metabolomics) were established to be substantial in toxicological research over the past decade. Currently, the impact of the AOP concept that tries to link adverse to molecular effects is the way forward to regulatory toxicology as proposed already by the Organisation for Economic Co-operation and Development (OECD), WHO and others. In risk assessment, however, ‘omics methods play a significant role in hazard identification, but not in risk characterisation due to the lack of relevant quantitative data on dose-response relationships. So, one goal in the future may be the integration of ‘omics data into the risk characterisation of chemicals.

Based on an example from food toxicology, it was shown how ‘omics data could be used in risk assessment and which way forward for their future role in risk assessment is possible. By using in silico methods (QSAR), mutagenic and carcinogenic chemicals were identified among more than 800 heat-induced processing contaminants and a priority list of compounds was established. Using this approach, 3-monochloropropanediol (3-MCPD) was identified. A detailed analysis of the proteome and transcriptome of 3-MCPD-treated rats identified molecular targets for 3-MCPD, e.g. related to glucose utilisation and oxidative stress. The antioxidant protein DJ-1 was strongly deregulated at the protein level in kidney, liver and testis, and giving new insights in the MOA of this relevant food contaminant. These new results were recently taken up by EFSA in the course of the risk assessment of 3-MCPD, showing that ‘omics data found its way into risk assessment in the MOA section. So far, there has been only limited use of ‘omics techniques in standard toxicity tests performed to identify adverse effects, because of existing limitations. However, by implementation of relevant MOA data into the AOP concept, it will be possible to link MOA effects observed by ‘omics methods to adversity. Furthermore, it will also be possible to develop appropriate in vitro test systems to predict adverse outcomes with significant evidence. Then, it may be possible to modulate the margin of exposure concept by comparing relevant human in vitro ‘omics data together with biological endpoints (AOP data) with human endogenic endpoints (metabolomics data, biomarker of exposure). In summary, new techniques such as in silico methods (e.g. QSAR, PBPK modelling) as well as ‘omics data together with endogenic biomarkers will fundamentally improve risk assessment in the future.

2.1.4. Integrating pharmacokinetics and pharmacodynamics in AOPs for next generation risk assessments

Quantitative analysis and modelling of data is one of the most important aspects of risk analysis and assessment. Relevant modelling activities are often divided into pharmacokinetics (PK; related to exposure assessment) and pharmacodynamics (PD; related to dose–response) in a rather simplistic way. We tend toward a fusion of the two disciplines into systems toxicology, at the point where they meet. In any case, modelling has always been important for low-dose extrapolation, exposure route adjustments or assessing the impact of interindividual variability. Yet, new challenges are emerging: quantitative in vitro to in vivo extrapolation, high-throughput and high content data integration, and
integration within the AOP framework. In response to the need for in vitro data integration and extrapolation, pharmacokinetic modelling has definitely taken a physiological (PBPK) turn in the last 10 years. Models of drug distribution of chemicals in the animal and human body have dramatically improved, but new models are now being developed to address the complexity of the new in vitro systems. The zebrafish model is usable for human and ecological risk assessments. A whole series of pharmacokinetic models of in vitro systems is also being developed in ongoing projects such as EU-ToxRisk. In parallel, the methods for fast simulations and calibration of complex models with experimental data have also been considerably improved over the last decade. AOP models are also being actively developed. Given their potential number and complexity, the best mathematical tools to use are not precisely now known. For extrapolation purposes, systems toxicology models would probably be favoured, being fundamentally mechanistic, like PBPK models. Yet, they can be extremely complex and data hungry. Statistical models (such as linked non-linear regression relationships or Bayesian networks) might be simpler to develop, but they may have more restricted applications. In between, there is a whole range of pharmacodynamic models, such as the effect compartment model, often used in pharmacology, but much less so in toxicology. Research is very active in those areas, and it is likely that, for a quite while, the various approaches will co-exist. PK/PD modelling of the effects of random mixtures of aromatase inhibitors on the dynamics of women's menstrual cycles was assessed. Using high-speed computer code, random exposures to millions of potential mixtures of 86 aromatase inhibitors present both in the US EPA ToxCast and ExpoCast databases were simulated. A PK model of intake and disposition of the chemicals was used to predict their internal concentration as a function of time (up to 2 years). In vitro concentration–inhibition relationships for aromatase were collected from ToxCast and corrected for cytotoxicity. The resulting total aromatase inhibition was input to a mathematical model of the hormonal hypothalamus–pituitary–ovarian control of ovulation in women. At aromatase inhibitor concentrations leading to over 10% inhibition of oestradiol synthesis, noticeable (eventually reversible) effects on ovulation were predicted. Exposures to single chemicals never led to such effects. However, a few per cent of the combined exposure scenarios were predicted to have potential impacts on ovulation, and hence fertility. These results demonstrate the possibility to predict large-scale mixture effects for endocrine disrupters with a predictive toxicology approach, suitable for high-throughput ranking and risk assessment.

2.1.5. Modelling tools to assess risks related to cadmium exposure for workers and consumers

This was a case study to provide a practical example of application of modelling tools to risk assessment. Specifically, the French Agency Anses (Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail) was asked to revise the dietary toxicological reference value (TRV) for cadmium and to propose cadmium maximum levels in fertilising materials and culture media to control soil pollution and in turn the contamination of plants for food use.

For non-smokers, food is the main source of cadmium exposure for its high environmental persistence and high rate of soil-to-plant transfer. Anses identified bone effects as the key effects and used the 2011 and 2012 epidemiological studies by Engström and colleagues (Engström et al., 2011, 2012) as the key studies for setting a TRV. The no-observed-adverse-effect level (NOAEL) for this effect for a population over 60 years of age corresponded to an internal dose of 0.5 μg cadmium/g urinary creatinine. Based on a PBPK model (Kjellström and Nordberg, 1978; Ruiz et al., 2010) that included data on the variation of creatinine excretion as a function of both age and body weight, and that related cadmium urinary concentrations to Cd oral concentrations, a tolerable daily intake (TDI) of 0.35 μg cadmium/kg body weight (bw) per day could be derived. The PBPK model also made it possible to estimate the urinary cadmium excretion limit (cadmium health-based guidance value (HGBV) in μg/g of creatinine as a function of age) not to be exceeded at any age to prevent exceedance of the internal TRV at adulthood, i.e. 0.5 μg/g creatinine at 50 years of age (Béchaux et al., 2014). Depending on the input of cadmium in soils (according to different scenarios of soil fertilisation), a predictive model was drawn up for estimating the trend of cadmium contamination in plants for human consumption over the following 99 years.

The construction of the model was carried out in two stages:

- Firstly, the transfer of cadmium from its input via fertilisers on agricultural soils to plant produce (potato and wheat grain) was modelled. This part of the model was built on the basis of a ‘mass-balance’ approach, taking into account: (i) all the routes of cadmium entry into the agricultural soil (fertilising materials, atmospheric deposition, irrigation water); (ii) routes of
cadmium release from the soil (food crops, leaching); (iii) variabilities; and also (iv) French specificities along this transfer. This first phase of the model made it possible to study the cadmium contamination of agricultural soils and crops as well as the cadmium leached, as a function of cadmium inputs via fertiliser materials and their agricultural practice in the next 99 years;

- In a second step, the transfer of cadmium through food from the plant produce to the consumers was modelled to estimate the impact on consumer exposure. Simulations of various fertilisation scenarios were run with an updated Anses model to predict changes in Cd concentration in wheat grain and potatoes. The obtained variations of the cadmium concentration in plants made it possible to estimate the impact on consumer cadmium exposure.

So, the prepared model based on cadmium flux is a predictive support to estimate cadmium levels in the plants and in the final related food products. The output data of the model allow derivation of the adult and child consumer's average chronic exposure and 95th percentile, as a function of the projection time of the modelling (10, 20, 60, 99 years), in correlation with the study of the trend of cadmium contamination in crops (wheat grain and potato) linked to fertilisation scenarios. It is also feasible to estimate a possible percentage of excess of the TRV. These mathematical models (from field to fork) are useful tools to support the risk assessment and decision-making processes. Based on such simulations, acceptable levels of cadmium pollution in fertilisers, soils and at the end food items may be determined.

2.1.6. Predictive tools in the risk assessment of new proteins in genetically modified organisms (GMOs): the case of coeliac disease

Coeliac disease (CD) is a disease of the small intestine characterised by flattening of the intestinal surface, resulting in a variety of clinical symptoms including malabsorption, failure to thrive, diarrhoea and stomach ache. The disease is caused by an uncontrolled intestinal CD4 T-cell response to gluten proteins in wheat (Triticum ssp.) and to the gluten-like hordeins and secalins in barley (Hordeum vulgare) and rye (Secale cereale). Oat (Avena sativa) is generally considered safe for patients although exceptions have been reported. The only available treatment is a life-long gluten-free diet including the exclusion of all food products that contain wheat, barley and rye or gluten and gluten-like proteins from these grains. CD has a strong genetic component as it is associated with particular immune response genes, called HLA in man (Koning et al., 2015). Most CD patients express certain HLA-DQ-molecules. HLA-DQ molecules are dimers of an alpha- (DQA1) and a beta- (DQB1) chain. As for all HLA-molecules, HLA-DQ molecules bind short peptides and present these to T cells of the immune system. The large majority of CD patients expresses HLA-DQ2.5 while the remainders are usually HLA-DQ8 positive. In patients, but not in healthy individuals, pro-inflammatory gluten-specific CD4+ T cells are present in the lamina propria of the affected duodenum. Importantly, these CD4+ T cells recognise gluten peptides only when presented by the disease associated HLA-DQ molecules. In essence, in patients with CD the immune system makes a mistake: the harmless gluten proteins in the food are recognised as if derived from a pathogen, leading to a pro-inflammatory response as long as gluten is consumed. Elimination of gluten from the diet constitutes an effective treatment as the T-cell stimulatory gluten peptides are no longer present. Unfortunately, once a gluten-specific T-cell response has developed, this results in immunological memory. Therefore, every subsequent exposure to gluten will reactivate the gluten-reactive T cells and results in inflammation. A life-long gluten-free diet is so required. T-cell epitopes derived from the \( \alpha, \gamma \) and \( \omega \)-gliadins as well as from the HMW and LMW glutenins have been reported. In addition, T-cell epitopes in both hordeins and secalins have been identified that are highly homologous or even similar to those found in wheat. A detailed knowledge of these known disease-causative sequences in gluten allows the design of a specific strategy to identify potential harmful sequences in other proteins. This strategy is presented in the EFSA guidance on allergenicity assessment of GM plants (EFSA GMO Panel, 2017).

2.2. New approaches in exposure assessment

2.2.1. Human biomonitoring

The European Human Biomonitoring Initiative (HBM4EU, https://www.hbm4eu.eu/) follows an innovative approach to generate the knowledge that policy makers need to improve policy in
environment and health. The over-arching goal of HBM4EU is to generate new knowledge, to inform the safe management of chemicals, and so protect human health in Europe (Ganzleben et al., 2017). Human Biomonitoring (HBM) data supply information on the aggregate exposure from all sources and by all pathways, serving as the basis to assess the risks from human exposure to chemicals. The research programme is based on the policy needs and priority chemicals identified after consultation with European and national policy makers. It builds upon existing knowledge from national and EU monitoring and research programmes. HBM4EU consists of more than 110 partner organisations from 28 countries, 27 European countries plus Israel, and is organised around 16 work packages led by key players of national HBM studies and research programmes. Major fields of activities are the science policy transfer, HBM studies and research to elucidate the impact of exposure on health. Data management under HBM4EU collects existing HBM data, currently fragmented in Europe. Exposure data valid for the whole of Europe, the identification of vulnerable or highly exposed subpopulations and the analysis of spatial and temporal exposure trends are major goals of HBM4EU. HBM is also considered to be key for addressing exposure to mixtures, as it reveals the extent and quality of multiple chemicals exposures. These data also demonstrate the need to develop concepts for health risk assessment beyond traditional single substance evaluation methods. Intensive communication with policy makers from the state of planning on will ensure that HBM4EU results are used in the further development and design of new chemicals policies, as well as in the evaluation of existing measures.

Among the recommendations for a better inclusion of HBM in risk assessment there are:

- the creation of awareness on capabilities of HBM at EU and national level;
- developing harmonised guidance for the use of HBM data;
- setting HBM HBGVs.

2.3. Holistic assessment of exposures to environmental and endogenous oestrogens by internal dosimetrics

Exposure to oestrogenic compounds through the diet and environment is an ongoing public health focus. This focus is based on a hypothesis that some endocrine-active compounds bind to oestrogen receptors to a sufficient degree to affect genomic signalling and so adversely impact normal endocrine function in animals and humans that, over time, leads to a number of diseases. For example, extensive research and risk assessment activity has centred on the potential for adverse effects from exposure to the food contact-associated oestrogenic chemical, bisphenol A (BPA), especially during the perinatal period. A large body of pharmacokinetic evidence from rodents, non-human primates and humans, which includes exposures during early neonatal and adult life stages, has been incorporated into PBPK models for BPA (Yang et al., 2015). Circulating concentrations of BPA in individuals with average and high consumption of canned foods are consistently in the low picomolar range. The modelled outputs for internal dosimetry from rodent and human models can also provide chemical-specific factors for use in computing HBGVs from toxicological studies in rodents. In addition, the plausibility of oestrogen receptor-mediated effects from BPA, based on measurements in serum and/or urine of BPA, dietary oestrogens [genistein (GEN), daidzein (DDZ)] and endogenous hormones [oestrone (E1), oestradiol (E2), oestriol (E3) and the fetal liver-derived oestetrol (E4)], was evaluated using mathematical calculations of fractional receptor occupancy (FRO) and relative responses (RR) for activation of oestrogen receptors (ERα and ERβ) in the presence of serum binding proteins (sex hormone binding globulin (SHBG) and albumin) in a cohort of pregnant women (Pande et al., 2019). These comparisons were made to critically evaluate the hypothesis that serum BPA must contribute sufficient added activity to shift total oestrogenicity by a meaningful increment over normal intraindividual daily variability to be considered important. The median FRO for BPA was five orders of magnitude lower than E1, E2 or E3 and three orders of magnitude lower than E4, GEN or DDZ. Similarly, based on the RR values, E3 was the most potent serum oestrogen during pregnancy (median RR values of 0.746 and 0.794 for ERα and ERβ receptors, respectively). The median RR values for E2 were 0.243 for ERα and 0.167 for ERβ. The RR values for the remaining oestrogens were consistently less than 0.01. Moreover, RR values were even lower for the dietary oestrogens, GEN and DDZ and BPA. Also, these minor interactions with BPA were dwarfed by the intraday and interindividual variability in the activity from endogenous oestrogens present in the pregnant women. Similarly, the receptor binding levels of endogenous oestrogens in normally cycling non-pregnant women suggest that BPA interactions would also be negligible. A consistent body of evidence comprising: (i) classical pharmacokinetic and PBPK modelling approaches that indicate minimal internal exposures from realistic doses; and (ii) the
implausibility of observable oestrogenic actions in ordinary pregnant women, reaffirms the conclusions of most regulatory bodies world-wide that exposure to BPA resulting from approved food contact uses is safe (US Food and Drug Administration, 2014; EFSA, 2015).

2.4. The exposome in practice

The identification of hazardous environmental pollutants is complex, particularly in relation to chronic, non-communicable diseases. The main contributors to this complexity are the diversity of hazards that may exist, the typically low levels of environmental contaminants/pollutants, long latency periods and largely unknown modes of action. The unravelling of environmental causes of disease is also limited by the technical difficulties in defining, and accurately measuring, exposures and by considerable spatial, temporal and intraindividual variation. The complex and partially unknown interaction with underlying genetic and other factors that modulate susceptibility and response to environmental exposures further complicates the process of delineating and understanding environmental hazards. To address such difficulties, the concept of the ‘exposome’ was proposed, initially by Wild (2005), with more recent detailed development in relation to its application to population-based studies (Wild, 2012). The original concept was expanded by others, particularly Rappaport and Smith (2010), who functionalised the exposome in terms of chemicals detectable in biospecimens. The exposome concept refers to the totality of exposures from a variety of sources including, but not limited to, chemical agents, biological agents, radiation and psychosocial component from conception onward, over a complete lifetime, and offers a conceptual leap in studying the role of the environment in human disease (Rappaport and Smith, 2010; Wild, 2012; Vineis et al., 2017).

There are two broad interpretations of the exposome concept, and they are complementary. One, called ‘top-down’, is mainly interested in identifying new causes of disease by an agnostic approach based on ‘omic technologies, similar to that applied in genetics with the genome-wide association study (GWAS) design. This approach is sometimes called an exposome-wide association study (EWAS), and utilises tools such as metabolomics or adductomics to generate new hypotheses on disease aetiology. The second general approach is called ‘bottom-up’ and starts with a set of exposures or environmental compartments to determine the pathways or networks by which such exposures lead to disease, i.e. which pathways/networks are perturbed. We have used the latter approach in the EU-funded EXPOsOMICS project (Vineis et al., 2017), that was focused on air pollution and water contamination. The experience of air pollution is particularly instructive. While in the 1970s and early 1980s air pollution was considered as a relatively marginal exposure in terms of attributable risks, the most recent estimate is that it accounts for 7.6% of global deaths and 4.2% of global disability-adjusted life years (DALYs) world-wide (Vineis and Fecht, 2018). The change in appreciation of the role of air pollution has been mainly due to the refinement of exposure assessment methods and the new generations of longitudinal studies. Mechanistic evidence via ‘omic technologies is now rapidly increasing, so lending credibility to previous epidemiological (‘black box’) associations (Vineis, 2018; Vineis and Fecht, 2018). In the EXPOsOMICS project, a few priorities for research were selected, with relevant practical implications for policy making and stakeholders: can our knowledge be consolidated on the health effects of those two important exposures, air pollution and water contaminants, reinforcing causal assessment? Can variation in exposures in a finer way than with the standard tools of epidemiology be detected? Can the effects of low and very low levels of exposure using ‘omic biomarkers be detected? How can ‘omic measurements to study pollutant mixtures be exploited? Can improved exposure assessment to calibrate estimates of risk and burden of disease be used? As a result, developed methodologies for the validation of a set of five ‘omics measured in the same subjects (for a total number of more than 2,000 individuals), and statistical tools to allow the analysis of very complex data sets were developed. Compared with air pollution, much less information is known about other environmental contaminants, some of which are widespread and pervasive, so suggesting the need for the same rigorous methods as those applied to air pollution.
2.5. Integrating new tools and new approaches in exposure assessment – examples

2.5.1. Integrated safety assessment of genetically modified food/feed: the experience from the EU projects GRACE and G-TwYST

The application of the classical tools developed for the risk assessment of chemicals to the evaluation of risk derived from GM plants has been very controversially discussed, particularly as regards animal studies on whole food and feed. The EFSA GMO Panel indicated the possibility to use a 90-day study on whole food/feed based on the OECD Test Guideline 408 (OECD TG 408, 1998) in case a specific hypothesis was identified in the course of the preliminary analysis of the GMO (e.g. comparative assessment of the compositional and agronomic-phenotypic characteristics of the GM crop) (EFSA GMO Panel, 2011). EFSA's Scientific Committee developed principles and guidance for the establishment of protocols for 90-day whole food/feed studies in rodents, adapting the existing OECD Test Guideline 408 to the peculiar test item 'food/feed' (EFSA Scientific Committee, 2011). Regulation (EU) No 503/20132 on applications for EU market authorisation of GM food and feed in accordance with Regulation (EC) 1829/20033 made mandatory the 90-day rodent feeding study on the whole GM food/feed for single transformation events, even in the absence of hypotheses. In a later explanatory statement (EFSA, 2014), EFSA provided further instructions on how to apply the general principles described in the EFSA Scientific Committee Guidance for the study design and analysis of such 90-day studies for GMO risk assessment and described two possible scenarios (scenario 1: a specific hypothesis is available, i.e. the preceding analyses have identified a potential risk(s); scenario 2: no specific hypothesis is available, i.e. no potential risk has been identified). Upon request from the European Commission, EFSA also prepared a scientific report that would support the future establishment of protocols for chronic toxicity and/or carcinogenicity studies in rodents with whole food/feed (EFSA, 2013). Two EU-funded projects, GRACE (GMO Risk Assessment and Communication of Evidence) and G-TwYST (Genetically modified plants Two Year Safety Testing), performed animal feeding trials and alternative in vitro methods with two different GM maize varieties to determine how suitable they are and what useful scientific information they provide for the health risk assessment of GM food and feed. Subchronic and chronic toxicity as well as carcinogenicity testing in rats was conducted based on OECD Test Guidelines for the testing of chemicals and on the above-mentioned EFSA documents. Under the GRACE project, 90-day feeding trials as well as a 1-year feeding trial with two GM MON810 maize varieties and several different near-isogenic varieties were performed (Zeljenková et al., 2014, 2016; Schmidt et al., 2017). Moreover, 'omics as well as in vitro (cell culture) approaches were performed to evaluate their possible added value in the overall risk assessment of GM crops (van Dijk et al., 2014; Sharbatli et al., 2017). Based on the EFSA explanatory statement, the OECD Test Guideline 453 as well as the scientific report by EFSA on the applicability of the OECD Test Guideline 453 to whole food/feed testing (EFSA, 2013) and taking into account possible concerns raised by a publication on the long-term toxicity of the GM maize NK603 (Seralini et al., 2012), the G-TwYST consortium performed two 90-day feeding trials as well as a combined 2-year chronic toxicity/carcinogenicity study in rats with the GM maize NK603. The main findings of the different experimental approaches in the GRACE and G-TwYST projects were presented. In these projects, it was concluded that rodent feeding trials do not provide added value to the risk assessment of GM crops in the case that relevant changes and/or specific hazards have not been identified in preceding analyses. Based on the experience gained in these two EU-funded research projects, it was highlighted that relevant aspects of the study design include the choice of the rodent strain, the incorporation rate of the GM crop on the intestine and on the immune system did not show changes in rats given the GM crops


as compared with concurrent controls. However, in the absence of a positive control, it is difficult to fully set out their relevance in this context. In the above-mentioned projects, attention was given to communication, transparency, engagement of stakeholders and Responsible Research and Innovation (RRI) principles.

2.5.2. CLARITY-BPA Project: Core NCTR/NTP study on BPA and lesson learnt on integrating regulatory and academic investigations in hazard assessments by the US National Toxicology Program

One of the challenges faced in the regulatory setting is the integration of data from investigative academic studies with those Good Laboratory Practice (GLP) studies conducted according to test guidelines for submission to regulatory agencies for making risk assessment decisions. The way in which these different types of studies are conducted and reported is a challenge when trying to integrate different data streams. These issues are often related to exposure levels, study design and conduct, chemical purity, statistical power, reporting of study details, selective reporting of data, risk of bias, directness of end-point measures to a health outcome, and data reporting transparency. One such a case is that of BPA. BPA is a chemical produced in large quantities for use primarily in the production of polycarbonate plastics and epoxy resins, that are used as lacquers to coat metal products such as food cans, bottle tops and water supply pipes. Human exposure to BPA is widespread, with 93% of Americans 6 years and older having detectable levels of BPA in their urine. The health impact of low-level exposure to BPA is a topic of considerable debate world-wide. On the one side a few GLP- and guideline-compliant studies support BPA safety at current exposure levels, on the other side hundreds of smaller scale research studies have indicated possible low-dose effects of this substance. In two subsequent presentations, the experience with the research programme developed by the US National Institute of Environmental Health and Safety (NIEHS), the National Toxicology Programme (NTP), and the Food and Drug Administration (FDA), the so-called Consortium Linking Academic and Regulatory Insights on BPA Toxicity (CLARITY-BPA; Schug et al., 2013) was presented. The CLARITY-BPA research programme was initiated with the aim of filling the gap between guideline-compliant research and hypothesis-based research projects on the toxicity of BPA (Birnbaum et al., 2013). This project investigated a broader range of potential health effects from exposure to BPA especially in the low-dose range that could inform regulatory decision making.

The CLARITY-BPA research programme has two components: (i) A ‘core’ modified guideline-compliant chronic study conducted at FDA’s National Center for Toxicological Research (NCTR) according to FDA GLP regulations (2-year perinatal only or chronic BPA exposure, including perinatal); and (ii) CLARITY-BPA grantee studies of various health endpoints, conducted by NIEHS-funded researchers at 14 academic institutions using tissues and animals born to the same pregnant rats and exposed under identical conditions as the core GLP study (Heindel et al., 2015).

In the core study, the toxicity of BPA administered by oral gavage from gestation day 6 until labour and then directly to pups by daily gavage from post-natal day 1 was examined in Sprague-Dawley rats. Study materials were monitored for background BPA levels throughout. A wide range of BPA doses was used ranging from as close as feasible to estimated human exposure levels to a reasonable margin of exposure (2.5, 25, 250, 2,500, and 25,000 µg/kg bw per day). Because many of the reported effects of BPA are associated with oestrogen-signalling pathways, two doses (0.05 and 0.5 µg/kg bw per day) of ethinyl oestradiol (EE2) were also included to monitor the response of the model to an oestrogen. In addition to animals dosed daily throughout the study (continuous-dose arm), a stop-dose study arm was included for the BPA doses only, with animals dosed until post-natal day 21 and then held without further treatment until termination, to assess any effects that were due to early exposure. In both study arms, animals were terminated at 1 year (interim) and 2 years (terminal). Statistical comparisons were conducted within sex, study arm, and sacrifice time and BPA and EE2 groups were analysed separately. Data collected included survival, body weights, litter parameters, age at vaginal opening, vaginal cytology, including an assessment of the onset of aberrant cycles, clinical chemistry (interim sacrifice only), sperm parameters (interim sacrifice only), organ weights (interim sacrifice only) and histopathology (both interim and terminal sacrifices). The grantee studies assessed a range of molecular, structural and functional endpoints that are not typically assessed in guideline-compliant studies (Heindel et al., 2015). As the safety assessment of BPA is outside the scope of this paper, for the results of the core and grantee studies the reader should consult the NTP website (https://manticore.niehs.nih.gov/cebssearch/program/CLARITY-BPA).
The key strengths of this consortium approach included: (i) the identical BPA exposure conditions used for both components of the consortium, which were provided at the same facility (NCTR); (ii) blinding of the core study samples received by the academic grantees, therefore minimising the potential risk of bias; and (iii) the development of an a priori list of endpoints to be collected per study and the requirement that all data be deposited in a private workspace in the NTP’s database before decoding. This allowed for confidential data acquisition and blinded deposition of data and also ensured that subsequent public access to data had no bias in end-point data acquisition. There were limitations to this approach though. Academic investigators were limited to using a specific shared design and model that may not have been optimal for the specific endpoints proposed. Sample acquisition was centralised and coordinated so highly specialised sample preparation or animal handling procedures required additional coordination, training and resources. Thirdly the scheme for peer review and selection of grantee proposals followed traditional National Institutes of Health (NIH) peer review procedures such that guidance was more general in nature and submitted proposals were not specifically aligned to address specific regulatory needs, but rather hypothesis generated research questions. Looking forward, a key lesson learnt from the CLARITY-BPA programme is that, for future initiatives, a less resource intensive approach is needed with much more targeted and integrated problem formulation and consortia development phase more direct communication between what the regulatory scientists need to make decisions and what the academic scientists can provide. This would result in closer alignment between the identified regulatory data gaps and the design of the studies and would maximise the utility of such collaborative programmes, while decreasing their costs.

2.5.3. Setting a health-based guidance value using epidemiological studies

In chemical risk assessment, the use of benchmark dose (BMD) for deriving a HBGV is increasingly being preferred over use of a single point estimate, such as the traditional NOAEL. In line with this development EFSA’s Scientific Committee (EFSA Scientific Committee, 2017) recommends that scientific panels should apply the BMD when setting HBGV. The use of BMD for human observational studies has been partly hampered by how findings from epidemiological studies are conventionally reported, highlighting the need for more dialogue between risk assessors and epidemiologists. More importantly, there is currently no consensus on how to derive BMD for human studies as the BMD methodology has mostly been developed for use in controlled studies in experimental animals. Although the same principles generally apply for human studies, existing guidance may not always be directly applicable. For example, in the updated EFSA guidance on BMD, model averaging based on a default set of pre-selected models is recommended. However, the recommended models do not include linear or other polynomial models. This may be logical for animal studies in which there are well defined unexposed controls (zero exposure) and the doses used often cover > 100 differences in exposure (making a linear response over the full exposure range highly unlikely). For human studies the observed exposure range is, in contrast, usually much narrower and the dose–response is often approximate linear. In addition, in observational settings ‘unexposed individuals’ (zero exposure) usually do not exist, making the reference point highly dependent on the study population and how it is selected. Furthermore, the high variability observed in human studies and the use of biomarker concentrations to assess exposure creates several additional challenges when deriving HBGV, and varying sample size the use of lower bound benchmark doses (BMDLs) for human data needs some careful considerations. In conclusion, there are no major obstacles for using human data to derive HBGV. BMD analyses can easily be performed, but existing conventions may not be directly applicable, and more work is needed (as has been carried out for animal data).

3. Conclusions and recommendations

A shift from the current risk assessment paradigm largely relying on animal studies to a more holistic approach is possible and desirable. Such a shift would be based on NAMs offering a better understanding of mechanisms leading to adverse effects and more accurate predictions of biological responses, helping to establish causality and waiving the use of animal studies. This would be integrated by novel approaches and tools in exposure and epidemiological sciences. Ultimately, this holistic approach would improve human health assessment while reducing animal testing. Envisaged by NASEM since 2007 and proposed in the EU in a SCHER/SCENIHR/SCCS report in 2013, such a change in human health risk assessment paradigm can already benefit from the availability of scientifically and technically robust tools and comprehensive data sets. Overall, this shift would help to optimise the way risk assessment is performed by both accelerating risk assessment pace and
embracing experimental models of greater human relevance, while addressing societal concerns about animal experimentation.

To support the paradigm shift to a holistic approach, efforts are needed, in the first instance, to promote the use of mechanism-based test systems, exploring molecular initiating events and early key events complementing or replacing experimental animals tests designed to show adverse effects. This is considered to be pivotal to support the understanding of biological pathways and would ultimately enable more accurate predictions of biological responses to single or multiple stressors and help to establish causality of effects. However, human-relevant in vitro assays, alternative models (e.g. zebrafish) and predictive modelling should be further developed and validated.

Efforts should be made to integrate NAMs with existing in vivo data matrices into existing AOPs but also to support the development of new ones. This would allow risk assessors to use AOPs informed approaches, to make sensible use of all available data, and to enhance confidence on the mechanistic understanding underlying a ‘regulatory’ adverse outcome. This could be achieved by fostering the causal link between molecular and cellular effects of substances, and their effects at the level of organs, organisms and populations.

The advances in new exposure and epidemiology sciences, as well as the availability of human biomonitoring data set should be considered, and strategies should be developed for their incorporation into a holistic exposure assessment. The use of already available data in the current risk assessment faces challenges. A rethinking of how to conduct and use epidemiological studies/data is also needed. These should be designed to be part of the path being incorporated in the AOP framework and used to consolidate human adverse outcomes in the testing paradigm.

Also relevant is the development of methodologies and guidance to refine the prediction of blood and tissue concentrations from exposure through TK or PBTK modelling. Conversely, in vitro-derived potency information needs to be converted into dosimetry information that, in turn, can be translated into corresponding external doses using in vitro–in vivo extrapolation (IVIVE) tools.

Data gathering, organisation, curation and use are perceived as priorities in this context. The development of databases and softwares for high-throughput screening (HTS) and high content image analysis (HCA) is needed to facilitate international harmonisation and promote the use of NAMs and the large amount of data produced by these technologies.

Strategies are required for re-engineering already available data matrices and making them accessible, readable, interpretable, usable and integrable with the new data streams. To facilitate and standardise a transparent risk assessment, electronic submission of toxicological, exposure and epidemiological raw data should be promoted by creating a digital network of relevant information.

The successful implementation of NAMs and other new developments in future risk assessment will necessitate cooperation of academia, risk assessors and international bodies such as the OECD, and the identification of these areas as high research priorities for long-term and flexible funding at the European level, this being supported by an effective dialogue between funding bodies, academia and risk assessors.

Overall, the recommendations from this symposium can be summarised as follows:

- bridging of the gap between traditional risk assessment methodologies, the assessment of non-standard endpoints and new approach methodologies to enable a shift in the risk assessment paradigm over the coming years;
- promoting the regulatory acceptance of reliable and predictive NAMs;
- provision of political support and long-term research programmes that are fit for purpose and that provide long-term and flexible research funding to make robust science available to accelerate the pace of risk assessment;
- promoting education and dedicated training programmes that engage risk assessment bodies, academia and relevant stakeholders to build the necessary capacity in terms of multidisciplinary expertise.

References


OECD (Organisation for Economic Co-operation and Development), 1998. Guideline for the testing of chemicals—repeated dose 90-day oral toxicity study in rodents, 408


Abbreviations

3-MCPD 3-monochloropropanediol
Anses Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail
AOP adverse outcome pathway
BDNF brain-derived neurotrophic factor
BMD benchmark dose
BMDL lower bound benchmark dose
BPA bisphenol A
bw body weight
CD coeliac disease
DALYs disability-adjusted life years
DDZ daidzein
DNT developmental neurotoxicity
EWAS Exposome-wide association study
FDA Food and Drug Administration
FRO fractional receptor occupancy
GEN genistein
GLP Good Laboratory Practice
GM genetically modified
GMO genetically modified organisms
GRACE GMO Risk Assessment and Communication of Evidence
G-TwYST Genetically modified plants Two Year Safety Testing
GWAS Genome-wide association study
HBGV health-based guidance value
HBM4EU European Human Biomonitoring Initiative
HCA high content image analysis
HTS high-throughput screening
IATA Integrated approach to testing and assessment
IVIVE in vitro–in vivo extrapolation
LOAEC lowest-observed-effect concentration
MOA mode of action


