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Chemical mixtures: challenges for research and risk assessment

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Rencontres scientifiques de l'Anses

Editorial

People are exposed every day and throughout their lifetimes to multiple chemical substances in the surrounding environment including food, water, air, soil and consumer goods.

In the last decades, the possible effects of chemical mixtures on humans and on the environment has become a major concern in many countries across the world. Both academics and regulatory agencies are increasingly addressing this challenge both in supporting research programmes and developing new methods to assess hazards and risk, including component-based approaches or whole mixture testing for the health effects associated with complex exposures.

Several recent reports and meetings in this field have highlighted research and expert assessment needs both in Europe (e.g. State of the art report on mixture toxicity, Dec. 2009, Kortenkamp, Backhaus, Faust; Opinion on the Toxicity and Assessment of chemical mixtures, SCHER, SCCS, SCENHIR, 2012) and in the US (e.g. Mixtures research at NIEHS, Nov 2012).

ANSES is organizing with National Food Institute, Technical University of Denmark and BfR a conference on the state of the art on chemical mixtures bringing together researchers and scientists from various disciplines (expology, toxicology, epidemiology, risk assessment...).

This conference will take stock of knowledge to better identify uncertainties and discuss research strategies and recommendations for expert assessment.

State of the art on chemical mixtures research

Sibylle ERMLER, Andreas KORTENKAMP

Brunel University, United-Kingdom

BIOGRAPHY

Sibylle ERMLER graduated in environmental biotechnology before conducting a PhD at the German Cancer Research Centre, Heidelberg. In 2007, she joined the Centre for Toxicology at the School of Pharmacy, London and moved to the Institute for the Environment, Brunel University, London, in 2011. Her research focuses on mixture toxicology and the implications of combination effects for human health with a particular interest in genotoxic mixtures and combination effects of endocrine disruptors.

ABSTRACT

Many chemicals are able to exert adverse effects on humans and wild life and exposure to such chemicals from food, consumer products and environmental media usually does not occur to individual compounds but to mixtures. Nevertheless, chemical risk assessment is traditionally carried out on a chemical-by-chemical basis, neglecting the potential for combination effects.

However, during the past 15 years research in mixture toxicology has made significant progress. This in turn led to an increased interest in considering whether the traditional chemical-by-chemical approach in chemical risk assessment should be augmented by including the risks of mixture effects.

This presentation will give an overview of the state of the art on chemical mixtures research and reflect on how this knowledge can be translated into risk assessment. Further, it will attempt to identify knowledge gaps that hamper the consideration of mixture effects in risk assessment.

The talk will address the question if a chemical-by-chemical approach could be justified if the definition of threshold doses or concentrations of regulatory concern and the introduction of uncertainty factors for individual compounds would safeguard against mixture effects. Evidence from experimental mixture studies showed that this might not be the case. Therefore, mixture effects should be given consideration in chemical risk assessment. As it is not feasible to test every conceivable combination of agents, one major aim of mixture toxicology is to utilise available data on single chemicals to predict their combination effects. Experimental data from human and eco- toxicological mixture studies showed that the concepts of dose addition and independent action provide good approximations of observed combination effects

Deviations from expected additive effects seldom occur, arguing for the utilisation of these concepts as pragmatic approach in chemical mixture risk assessment. The presentation will give an overview of the progress in chemical mixtures research during the last decade and end with an outlook on current and future developments.

Session 1
How far are we in research on exposures?

The Exposome: a new concept to support research on causes and prevention

Christopher P. WILD
International Agency for Research on Cancer, France

BIOGRAPHY

The main research interest of Christopher P. WILD is to understand the interplay between environmental and genetic risk factors in the causation of human cancer. He particularly seeks to apply biomarkers in population-based studies to this end. He was Professor of Molecular Epidemiology at the University of Leeds from 1996 and Director of the Leeds Institute of Genetics, Health & Therapeutics before being appointed as Director of the International Agency for Research on Cancer (WHO/IARC, Lyon) in 2009.

ABSTRACT

Population demographics of aging and growth will result in 70% more new cancer patients every year by 2030, with the greatest increases in low and middle-income countries. In many such countries access to cancer therapy remains limited and personalized medicine is unlikely to make great inroads in the foreseeable future. It is implausible that the world will be able to treat its way out of cancer. Therefore a high priority must be placed on prevention in order to combat this impending epidemic.

Identification of risk factors is one foundation for cancer prevention. Much is known already, with typical estimates of 30-50% of cancers preventable based on current knowledge. However, there remain a number of common cancers for which the aetiology remains obscure (e.g. prostate, pancreas, kidney, brain and haematological cancers) and others where only a proportion is explained (e.g. colorectal, oesophageal and breast). In some cases interventions need to be evaluated while for others there are barriers to implementation of the available interventions. The role of environmental contaminants is has been particularly difficult to address in relation to cancer risk but also is an area where regulation can be successfully applied to reduce human exposure, if the evidence-base is available to support such action.

Advances in understanding the human genome are being translated into improved therapeutics, tailored to exploit the underlying tumour genetics of an individual patient. This personalized, or stratified, medicine promises improved survival for patients with tumours amenable to this therapy and for which there is a commercial imperative for drug development. However, there are also great opportunities to be derived from application of these new molecular technologies to epidemiology (Wild 2012a).

First, biomarkers promise improved measurement of environmental and lifestyle exposures. This includes the possibility that whole-genome or exome sequencing will reveal clues to the aetiology of cancer, including in relation to the role of environmental chemical contaminants. Second, molecular data across different "omics" platforms provide comprehensive portraits of cancer sub-types. Such molecular profiling allows exposure to be analysed in genetically-defined sub-sets of cancers, possibly revealing new underlying associations. Third, recognition that exposures may act through epigenetic alterations is leading to new paradigms, biomarkers and fresh opportunities to investigate the biological plausibility of exposure-disease associations.

Fourth, biomarkers may provide valid surrogate endpoints in evaluation of interventions. Finally, molecular tools will allow exposures throughout the life-course (including the perinatal period) to be linked to biological changes that may provide clues to subsequent cancer risk.

The above areas are promising ones where laboratory sciences may be applied to epidemiology. However, for major advances to be made, a number of critical changes are needed: 1) develop and promote inter-disciplinary research, underpinned by appropriate training; 2) policymakers and funders to prioritise translational research into cancer prevention, recognizing that investment in this area is less attractive for the private sector than clinical research; 3) clear advocacy to illustrate to the public, politicians and non-governmental organizations the limitations of improved treatment in addressing the growing global cancer burden.

In summary, translational cancer research stands at an exciting but critical point in time. What is needed is a concerted effort to drive the advances in basic science towards prevention of cancer and other noncommunicable diseases, with an eye to reducing global inequalities in health in the process (Wild 2012b).

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The ACROPOLIS project in summary

Jacob D. VAN KLAVEREN, PE. BOON, R. GLASSS, A. MORETTO, W. VERBEKE, F. ROSSENEU and H. V.D. VOET

National Institute for Public Health and the Environment (RIVM), The Nederlands

BIOGRAPHY

Jacob VAN KLAVEREN had his education in Human Nutrition at Wageningen University. He became an advisor for the national government and international bodies like the WHO and EFSA. He coordinated European projects and the RTD-programme regarding food safety within the Wageningen University and Research centre. Presently, he works at the Dutch National Institute for Public Health and the Environment as senior scientific advisor for integration of models in public health and risk assessment.

ABSTRACT

The overall objective of the ACROPOLIS project is to improve risk assessment strategies in Europe. The project has developed a framework for cumulative and aggregate risk assessment of pesticides that is scientifically sound and accessible for all actors involved in the European risk assessment and risk management. The specific objectives of the project are:

1. improved cumulative exposure assessment methodology;
2. new models for aggregated exposure assessment addressing different routes of exposure;
3. setting up new toxicological testing for identifying possible synergistic effects and developing a strategy for refinement of cumulative assessment groups;

4. to integrate cumulative and aggregate risk models in a web-based tool, including accessible data for all stakeholders;
5. improving the understanding of cumulative risk assessment methodology of different stakeholders.

The framework of probabilistic exposure assessment has been set in the EFSA guidance on the use of probabilistic modelling for dietary exposure to pesticide residues. We examined the practicality of performing a cumulative dietary exposure assessment according to the requirements of the EFSA guidance. For this, the cumulative exposure to two Cumulative Assessment Groups (CAGs) of triazole pesticides was estimated using national food consumption and monitoring data of several European countries.

EU member states perform annually analyses of pesticides on raw agricultural commodities (RACs) intended for human consumption to monitor the occurrence of pesticide residues. The food consumption data were obtained from the Comprehensive database compiled by EFSA. We also organised data for processing effects based on the BfR database and additional information from Draft Assessment Reports. The observed data gaps were filled and additional data as required by the EFSA guidance was added to the input database.

Application of the optimistic model run, as proposed in the EFSA guidance is feasible, however, resulting exposure estimates are underestimates of the real exposure. The pessimistic approach overestimates the risk. Especially the inclusion of MRLs of animal commodities seems to result in unrealistic conclusions regarding the contribution of animal commodities to the dietary exposure.

Both optimistic and pessimistic runs were feasible. A possible conclusion may be that some kind of intermediate 'realistic' scenario is needed that combines the optimistic and pessimistic model run in such a way that it results in more realistic exposures. These exposures can still be argued to be conservative (precautionary principle) but not over-conservative as now seems the case with the pessimistic model run.

The project has performed a qualitative and quantitative analyses of stakeholder's attitudes, understanding and willingness to accept new advanced models. ACROPOLIS' perceived relevance and expected outcomes are evaluated favourably. Apart from practicalities to get external data connected and open issues for risk management, the cumulative ACROPOLIS model is well-received throughout Europe and many member states and stakeholders are now making use of the ACROPOLS cumulative model. The model is validated, transparent and fully documented. The (complex) model exercise, including all uncertainty analyses, can be performed via Internet, which is a novelty. However, it is a still a proof of principle, which needs further testing once common assessment groups become larger or when exposure to mixtures needs to be applied with the full European data collection on a routine basis.

Aggregate exposures combine dietary and non-dietary sources including a single and multiple compounds approach. Examples of aggregated exposures are occupational farming activities, use of amateur or consumer products, or incidental exposures experienced by residents or bystanders who are all persons also exposed to pesticides via consumption. We implemented conceptual framework of aggregated exposure into software and we tested the software addressing four different aggregated exposure scenarios in the form of case studies. Further testing is recommended.

The ACROPOLIS project has also resulted in two '*in vitro*' test systems and two PB-PK models. *In vitro-in vivo* extrapolation is necessary to express the dose-response for *in vitro* data on a similar dose scale as the *in vivo* data. In order to do this, the application of PB-PK modelling, when enough data are available can be very useful to extrapolate from the *in vitro* to the *in vivo* situation in animals, and possibly from animals to humans.

In the context of CRA, in vitro studies as performed in the ACROPOLIS project have been proven to be useful for both refinement of grouping and confirmation of the dose-additive assumption.

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Identifying mixtures from combined exposure as a first step towards hazard characterization

Amélie CRÉPET

ANSES, French Agency for Food, Environmental and Occupational Health and Safety, France

BIOGRAPHY

Dr. Amélie CRÉPET has a PhD in applied mathematics to food risk assessment. She has worked in this field since 2002 and at ANSES since 2007. Amélie CRÉPET has developed probabilistic and Bayesian models related to food risk analysis for general and specific groups of consumers aimed at improving knowledge of exposure modeling and risk assessment to environmental contaminants, pesticides residues, pathogenic bacteria and food allergens.

She has experience in coordinating research programmes: the national PERICLES (ANR 2009-2012) project on exposure to mixtures of active substances and their possible combined effects on human cells and a national project on risk assessment to food allergens called MIRABEL (ANR 2011-2014).

ABSTRACT

Due to the large number of chemicals found in the environment, individuals are everyday exposed to complex chemical mixtures which can interact. Current risk assessments are made for single substances and therefore do not consider these interactions. One reason lies in the multitude of possible chemical combinations for which it is unrealistic to test combined toxicological effects. However, several guidelines have been recently published to assess the risk for mixtures (US-EPA 2007, EFSA 2013a, 2013b). The issue is that these guidelines most often recommend to group substances from a same chemical family. Therefore, they are exclusively focused on the hazard and do not include the exposure in the mixture selection. This makes it impossible to study chemical mixtures to which humans are actually exposed. It is a fact that individuals are simultaneously exposed to different chemicals: either through one food, through food combination at a meal, or even through meal combination during a lifetime. That is why identifying the major combinations should be a first step, before assessing the hazard of these mixtures.

The purpose here is to present original statistical methods recently developed to assess the chemical mixtures to which the French population is exposed in their diet. A first approach consists in working directly on exposure data (Crépet, et al. 2011; Crépet, et al. 2013a). The exposure to the different chemicals is modeled to cluster individuals into subgroups with similar combined exposure profiles. Then, for each subgroup, the exposure correlations between chemicals are studied to define the mixtures. A second approach is to first define subgroups of individuals from their consumption patterns (Béchaux, et al. 2013). Then, consumption patterns are combined with the residue levels to link dietary behavior with exposure to chemical mixtures. This approach makes possible for the characterization of the potentially high-risk subpopulations. It makes it easy to interpret the mixtures through the individuals' diet which brings useful results for risk management. A third approach is to add hazard information in the process of mixture identification. The values and limitations of these approaches will be investigated.

These approaches will be applied to two sets of data: the 79 pesticide residues used in the PERICLES research program (Crépet, et al. 2013b) and 149 chemical substances coming from the Second Total Diet Study (Sirot, et al. 2009).

This work will also lead to tackle the concept of dynamic exposure modeling, extend the physiologically based pharmacokinetic (PBPK) models for mixtures, and integrate biomonitoring data in combined exposure assessment.

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Development of methodologies to be used for assessing exposure to multiple pesticides

Hermine REICH

Pesticides Unit, European Food Safety Authority, Italy

BIOGRAPHY

Hermine REICH is working for the European Food Safety Authority (EFSA) in Parma (Italy) as senior scientific officer in the Pesticide Unit. In this post she is responsible for the implementation of Regulation 396/2005, in particular the risk assessment of pesticide residues in the framework of setting legal limits for residues in food, a review programme for maximum residue levels (MRLs) the preparation of the annual report of pesticide residues found in food controls and risk communication.

She has graduated in biotechnology and food technology and throughout her whole professional career she was responsible for different aspects in the risk assessment, risk management and risk communication of pesticides.

ABSTRACT

According to the WHO methodology and the risk assessment approach used at EU level in the framework of pesticide authorisations and MRL setting, the dietary exposure to pesticide residues is calculated for each individual active substance separately.

However, Regulation (EC) No 396/2005 acknowledges that consumers are expected to be exposed to multiple residues present on food eaten with one meal, during one day or over a longer period which may lead to cumulative (additive or synergistic) effects on human health. Since no internationally agreed methodology

is available to assess the cumulative exposure, EFSA has worked intensively on this subject.

The main focus of the activities during the last years was on the establishment of cumulative assessment groups. So far the work on the assessment groups regarding pesticides which have an effect on the nervous system and on the thyroid has been completed. The assessment as regards effects on the liver, reproductive system, adrenal, eyes and kidney will follow.

However, to implement cumulative risk assessment in the routine MRL setting process, work on different areas need to be completed which requires a close collaboration of risk managers and risk assessors to ensure that the assessments are addressing the practical needs. On the side of risk assessors, an interdisciplinary approach is required, bringing together expertise in toxicology, food consumption, pesticide dietary exposure assessment, pesticide monitoring and in development of calculation models.

A important work package in the cumulative risk assessment project is to find an agreement on the food consumption data that should be used for the calculation of the cumulative dietary exposure. Therefore negotiations with the owners of the European food consumption data are required which aim to get an agreement that consumption data generated at Member State level can be used for this purpose. Overall, it needs to be ensured that sufficient consumption data are available which are representative for all relevant subgroups of the European population. Since food survey data were generated according to non-harmonised protocols and the results are reported in different ways, it is necessary to convert the consumption data to make them comparable and matching with the food classification which is used as the basis for MRL setting. EFSA also proposed that for the first tier calculation, consumption data which are reported for processed commodities should be re-calculated to the corresponding unprocessed commodities. This manipulation is expected to make the lower tier cumulative exposure calculations more conservative, but without this recalculation the lack of monitoring data for processed commodities might underestimate the actual exposure.

This brings us to the second major work package which concerns the field of monitoring data. To ensure that sufficiently representative monitoring data are available which allow a reliable estimation of the background exposure for the cumulative assessment groups, the EU coordinated monitoring programme should be adapted, taking into account the active substances that were included in the cumulative assessment groups. From indicative calculations performed in the framework of the Annual Report on Pesticide residues it became also evident that there is need to find a practical solution how to handle the so-called "non-detects" - these are monitoring results below the limit of quantification - since the high number of non-detects is increasing the overall uncertainty of the calculations.

The consumption data and the monitoring results have to be combined in a practical calculation tool. The aim is to make a risk assessment tool available to be used by EFSA, applicants, Member State experts responsible for consumer risk assessment and which is also shared with other stakeholders who have an interest in consumer risk assessment for pesticides. Preferable, a simple screening tool should be developed which is easy to handle but sufficiently conservative. For cases where more refined calculations are needed, the use of probabilistic calculation models is certainly the state of the art.

EFSA also expects that during the practical implementation period the need for further adaptations of the methodology will be identified. Thus, the method development is an iterative process taking into account the practical experiences.

Session 2 – How far are we in research on toxicology?

Analysis of mixture effects of (tri)azole fungicides in a broad dose range ex vivo and in vitro with special focus on liver

Tanja HEISE

BfR, Germany

BIOGRAPHY

She studied biology at the Free University in Berlin (Germany) and received her PhD at the Institute for Microbiology at the TU Braunschweig. Since 2007 she has been working at the Federal Institute for Risk Assessment (Berlin). There she developed a hepatic in vitro system for the detection of liver carcinogens at the department Food Safety; at the department Chemicals Safety we started in 2011 a pilot project for the investigation of combination effects of multiple residues of pesticides.

ABSTRACT

Consumers are exposed to multiple residues of different pesticides via the diet. This raises questions concerning potential cumulative effects. Since substances are tested for regulatory purposes on an individual basis at generally high dose levels, there is only limited data available on potential mixture effects especially in the low dose range. Hence, the analysis of combined effects of substances in a broad dose range represents a key challenge to current experimental and regulatory toxicology. Among the active substances which are most frequently used and for which combination effects due to a similar mode of action seem plausible, a group of fungicides and antifungal drugs, the triazoles, play a prominent role. Triazoles are widely used as pesticides to protect plants from fungal diseases such as *Septoria tritici* or *Fusarium* infections and as drugs to treat systemic and local fungal infections such as candidiasis or aspergillosis. Triazoles are designed to inhibit a specific fungal cytochrome P450 enzyme, CYP51 (lanosterol-14-α-demethylase). Due to the ability to bind to the heme protein and inhibit CYP-dependent enzymes, triazoles are known to effect hormone synthesis and drug metabolism. To analyse potential mixture effects, four triazoles and an imidazole (prochloraz) were administered at first individually to rats in a 28-day feeding study. A broad dose range was chosen covering a typical toxicological threshold level (based on a no observed adverse effect level (NOAEL) derived from regulatory studies), a level near to the reference dose (NOAEL/100) as well as a clear effect dose (NOAELx10). Since these fungicides are considered to cause liver toxicity by a mechanism involving the constitutive androstane receptor (CAR), a known CAR activator (phenobarbital) was also administered. Based on the results of the experiments with single substances, cyproconazole, epoxiconazole and prochloraz were subsequently chosen for the analysis of mixture effects. Test parameters where those usually required in guideline-conform toxicological studies such as body weight and food consumption, clinical chemistry, organ weights and histopathology, but state-of-the-art molecular toxicological methods were also applied to analyse e.g. alterations in gene expression. Our results show a dose dependent increase in liver weights and concomitant histopathological changes (vacuolisation, hypertrophy) at the upper dose levels only. For most parameters affected the results imply monotonic dose response curves and confirm the NOAELs derived from regulatory studies when the 'classical' toxicological parameters are considered. However, the expression of sensitive marker genes was significantly affected also at the intermediate (around NOAEL and NOAEL/10) and at the lowest dose levels (NOAEL/100).

With regard to these detected effects on the molecular level at doses below the NOAEL, it is not possible to define a threshold or no effect level for all endpoints, although a clear distinction between adverse and adaptive outcomes is not feasible before a pathway-focussed analysis concerning the biological relevance of the results has been performed. Nevertheless, the need to consider molecular effects and the relevance of such changes for regulatory toxicology should be discussed.

In addition, we evaluate if the effects of triazoles and especially combination effects can also be detected *in vitro*. As the results of the *in vivo* study confirmed that triazoles show mainly hepatotoxicity and to a lesser extent endocrine disruption with the adrenals being the main endocrine target organ, cell lines from human liver and an adrenal cell line are used. Since triazole dose-levels were measured in selected target organs at the end of the 28-day feeding study, concentrations used for the *in vitro* experiments shall reflect realistic exposure concentrations. This approach ensures that the parameters analysed *in vitro* reflect the physiological conditions in vivo with respect to the substance concentrations, target organs, their molecular targets and their toxicokinetics and will show if an identification of mixture effects *in vitro* is possible.

A Concentration Addition Model to Assess Activation of the Pregnane X Receptor (PXR) by Pesticide Mixtures Found in the French Diet

R. RAHMANI, G. de SOUSA, A. NAWAZ

XCMT, INRA UMR 1331, Research Centre in Food Toxicology, France

BIOGRAPHY

Dr. R. RAHMANI is Research Director at INRA and head of the Xenobiotic's Cellular & Molecular Toxicology team (*UMR 1331*). He is expert in: -*in vitro* xenobiotics metabolism & toxic mechanisms; -assessment of hazards & risks associated with drugs, dietary or environmental chemicals; -studies on cell signaling pathways involving detoxi-fication systems & cell death regulation, through nuclear receptors. He has coordinated numerous National, European or industry-based research programs & is member of National and International Toxicology expert comities.

ABSTRACT

The estimation of health risks linked to pesticides is widely based on toxicological data and reference values for the substances taken individually. These compounds are however generally present in foods in the form of mixtures of residues that, depending on their concentrations, metabolism and modes of action, may have additive, supra-additive, infra-additive, synergic or antagonistic impacts on human health. Therefore, interactions linked to multiple pesticides exposure are not taken into account in risk evaluation, notably in cases of molecules exhibiting similar mechanisms of action, such as nuclear or hormones receptor activation.

Among those receptors, the PXR regulates the transcription of genes encoding xenobiotic detoxication systems (phase I and II biotransformation enzymes, transporters, cell survival...) and evidence also exists that it has an endobiotic function that can impact cell metabolism and energy homeostasis. While we know that PXR has broad ligand specificity and can be activated by large numbers of pesticides, how it is activated by pesticides present in a mixture remains yet unclear. Based on the exposure of the French population to pesticides through their diet (PERICLES program), we therefore tested the human PXR transactivation potency of seven mixtures, using a stable hepatoma cell line expressing luciferase under hPXR control (HepG2-hPXR). The three cocktails with the highest transactivation potency were evaluated using the concentration addition model. The potency of the individual pesticides as well as their mixtures (both at equimolar or in real-life exposure proportions) were tested at seven concentrations from 0.1 to 100 µM. PXR activation was modeled (five-parameter curve) using the bootstrap method. We applied the concentration addition model to the whole data set generated after bootstrapping to obtain the uncertainties of the predicted dose-response curves. Of the fourteen pesticides included in our study, only four were found to be full agonists. The concentration addition model could predict the effect of all three mixtures up to 5 µM.

This model predicts the full dose-response of a mixture only if it is composed mainly of full agonists. Data obtained with HepG2-hPXR were also well correlated to human hepatocytes data (induction of CYP_3A_4 expression, under PXR control). On the whole, our results demonstrate that hPXR activation represents a useful tool for assessing the health risk of xenobiotics mixtures and indicate the need to reconsider the pesticide legislation which is mostly based on active substances taken individually.

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Mixture effects of endocrine disrupters – severe and predictable

Ulla HASS, Sofie CHRISTIANSEN, Marta AXELSTAD, Julie BOBERG, Anne Marie VINGAARD, Pernille R. JACOBSEN, Louise K. ISLING

Division of Toxicology and Risk Assessment, National Food Institute, Technical University of Denmark

BIOGRAPHY

Professor in reproductive toxicology and leader of the research unit Reproductive Toxicity Group, National Food Institute, Technical University of Denmark.

Main work tasks: Research in reproductive toxicology, especially on mixture effects of endocrine disrupters in rats, and scientifically based advice to regulatory bodies.

Extensive experience with regulatory use of in vivo data in e.g. REACH and OECD.

More than 50 papers in peer reviewed journals and many scientific reports for regulatory bodies.

ABSTRACT

Risk assessment of chemicals is generally based on a comparison of human exposure levels to an experimental NOAEL (No Observed Adverse Effect Level).

This is in most cases done for one chemical at a time, but humans may daily be exposed to many different chemicals.

Several endocrine disrupting chemicals have for example been detected in mixtures in humans, incl. children. This raises important questions: Can combined exposure to endocrine disrupting chemicals induce severe effects, although the dose levels for the individual chemicals are around or below their NOAELs and can these effects be predicted?

We have designed large experimental mixture studies in rats to assess whether combination effects occur when chemicals are combined at doses sufficiently low to be without observable effects when tested on their own. Often, these doses were in the range of those commonly used to derive estimates of safe human exposures (so-called points of departure, usually NOAELs, or benchmark doses).

For combinations of chemicals that interact with the same molecular target, there is clear evidence that mixture effects can arise at doses around, or below, NOAELs. Also, the mixture effects can be predicted based on dose-addition.

There is also good evidence that combinations composed of chemicals with diverse modes of action but similar effects induce mixture effects when each component is present at doses equal to, or below NOAELs. We have for example investigated the effects of mixtures of a widely used plasticizer, di(2-ethylhexyl) phthalate (DEHP); two fungicides present in food, vinclozolin and prochloraz; and a pharmaceutical, finasteride, on landmarks of male sexual development in the rat, including changes in anogenital distance, retained nipples, sex organ weights, and malformations of genitalia. These chemicals were chosen because they disrupt androgen action with differing mechanisms of action. Surprisingly, the effect of combined exposure on malformations of external sex organs was synergistic, and the observed responses were greater than would be predicted from the toxicities of the individual chemicals. In relation to other hallmarks of disrupted male sexual development, including changes in AGD, retained nipples, and sex organ weights, the combined effects were dose additive. When the four chemicals were combined at doses equal to their NOAEL, significant reductions in AGD were observed in male offspring.

We have also studied the effects of mixtures modelled based on human intakes. To address this issue for the first time, we selected 13 chemicals where data about in vivo endocrine disrupting effects and information about human exposures was available, including phthalates, pesticides, UV-filters, bisphenol A, parabens and the drug paracetamol. The mixture ratio was chosen to reflect high end human intakes. The results suggest that highly exposed women of reproductive age may not be protected sufficiently against the combined effects of chemicals that affect the hormonal milieu required for normal sexual differentiation of fœtuses.

In conclusion, the chemical-by-chemical approach in risk assessment appears as insufficiently protective against the possibility of mixture effects and there is a need for changes in the current practice. In most cases the mixture effects were dose additive. Thus, cumulative risk assessment for endocrine disrupters is feasible and dose addition as an assessment method is recommended as a default, until evidence as to the suitability of alternative assessment concepts emerges. Using molecular mechanisms of action as the starting point for grouping of endocrine disrupters into classes to be subjected to cumulative risk assessment appears insufficient and instead grouping criteria should focus on common adverse health outcomes and the likelihood of co-exposures.

Exposure to pesticides alone or in combination: biomarkers and effects in vivo and in vitro approaches

C. DEMUR[1], C. CANLET[2,3], B. MÉTAIS[2,3], F. BLAS-Y-ESTRADA[2,3], C. SOMMER[2,3], L. GAMET-PAYRASTRE[2,3]

[1]Laboratoire d'hématologie, Pavillon Lefebvre, CHU Purpan, France - [2]Inra, Toulouse University, UMR1331, Toxalim, Research Centre in Food Toxicology, France - [3]Toulouse University, INP, UMR 1331, Toxalim, France

BIOGRAPHY

Researcher at the National Institute of Agronomic Research, Laurence GAMET-PAYRASTRE works at the Research Centre in Food Toxicology in Toulouse (France). She is interested in the impact of dietary exposure to low dose pesticides mixture and she has coordinated 2 national projects supported by ANR and ANSES on this research thematic. She was also involved in an international project PHC IMHOTEP to study the transfer of pesticide residues to mice offspring upon prenatal exposure and the ameliorating effect of anti-oxidant.

ABSTRACT

Consumers are exposed to a mixture of pesticides through their food intake. These compounds are considered risk factors for human health, and the impact of dietary exposure to low doses of pesticide mixtures remains poorly understood.

For this study we first developed a mouse model to mimic consumer exposure in order to compare the effect of pesticides both alone or combined at doses corresponding to their Acceptable Daily Intake value. Female mice were exposed to pesticides throughout gestation and lactation. After weaning pups were fed the same pesticide-enriched diet their mothers had received for an additional 11 weeks. We assessed metabolic changes in the plasma of offspring using a 1-H NMR-based metabonomic approach and investigated the impact on hematopoiesis by counting peripheral blood cells and performing bone marrow assays *in vitro*. In parallel we investigated the impact of a chronic exposure to pesticide mixtures on a representative *in vitro* model for human intestine since this tissue comes into direct contact with food contaminants *in vivo* (Colonic adenocarcinoma Caco-2 cells). Cells were allowed to grow and differentiate in the presence of chlorpyrifos, atrazine or endosulfan both individually or in combination at very low doses (ranging from 0.1 to 10 µM). Impact of such an exposure was assessed on cell viability, DNA damage, cell differentiation and integrity of intestinal barrier.

Metabonomic analysis of mice plasma showed a specific metabolic fingerprint for each pesticide exposure in adult offspring. Interestingly metabolite differences were observed as early as weaned animals that had not yet been directly exposed themselves. Studies of the hematopoietic system revealed that dietary exposure to one particular pesticide, endosulfan, produced a significant decrease in red blood cell and hemoglobin levels, consistent with hemolytic anemia. Cell signaling profiles of bone marrow progenitors were also clearly affected. Moreover, we found that dietary exposure to a mixture of pesticides had effects that differed according to the targeted organ or tissue. The effects of pesticide mixture on haematopoiesis were often lesser or equal to that of the most efficient pesticide (endosulfan). On the opposite the intensity of epileptic discharges experimentally induced by 30µM 4 aminopyridine (4-APantagonist K+ channel) in hippocampal slices were increased only in animal exposed to the mixture whereas no change was observed in animal exposed to pesticide alone.

In Caco-2 cells, chronic exposure to the individual pesticides at very low doses led to significant perturbations that could damage intestinal epithelial cells and alter their barrier function. A 14-day low dose of endosulfan alone significantly decreased cell viability and increased DNA damage associated with a decreased expression of cell membrane ABC transporters and increased membrane resistance. Exposure to chlorpyrifos which also appeared genotoxic at 10µM led to an increase in mRNA expression of the BCRP and P-gp ABC transporters. In this in vitro model, the pesticide mixture induced significant genotoxicity which did not result from an additive or a synergistic effect of pesticides and on the contrary to that observed upon exposure to pesticide alone, the mixture did not significantly affect the expression of membrane transporters, the differentiation process or the integrity of the epithelium layer.

In conclusion, our in vivo and in vitro results suggest that the effect of pesticide mixtures cannot be predicted from the combined effects of their constituent compounds and depend on the targeted tissue.

This work was supported by a grant from ANR (Expomatpest) and ANSES (Epicee).

Mixture effects at human relevant exposure levels?

Niels HADRUP, Mikael PEDERSEN, Kasper SKOV, Line OLRIK BERTELSEN, Kristine GRØNNING KONGSBAK, Henrik FRANDSEN, Julie BOBERG, Ulla HASS & Anne Marie VINGAARD

Division of Toxicology and Risk Assessment, National Food Institute, Technical University of Denmark

BIOGRAPHY

Professor Anne Marie VINGAARD conducts research at the National Food Institute, Technical University of Denmark and has >20 years of experience within toxicology with a focus on mechanisms of toxicant action. The group's special field of expertise is endocrine activity of chemicals and the team is engaged in cocktail effects of chemicals, obesity development, and development of computational tools to predict toxicity. Presently, she heads a comprehensive project for the Danish Food Ministry aiming at providing tools for the authorities for risk assessment of cocktails of chemicals in food.

Anne Marie VINGAARD was recently ~4 years in a pharmaceutical company, developing a strategy for early toxicity testing.

ABSTRACT

It is well-known that mixtures of endocrine active chemicals usually act additively irrespective of the applied model system and the complexity of the endpoint measured. Only in rare cases synergism or antagonism occurs. Most studies performed so far have tested doses at or above the NOAEL for the individual chemicals. But what happens if we test mixtures of EDC's in rats at doses close to human relevant exposure levels?

We had the hypothesis that a low-dose mixture of environmental chemicals and active food ingredients would not affect toxicity induced by perfluornonanoic acid (PFNA).

PFNA was given at 12.5, 250 or 5000 µg/kg/day for 2 weeks to adult male rats with or without a mixture containing 14 chemicals including phthalates, bisphenol A, parabens, UV filters, pesticides, and the CYP3A4 inhibitors bergamottin (from grape fruit) and glabridin (from liquorice) at human relevant exposure levels (called 'cocktail' at a dose of totally 2.5 mg/kg/day). The lowest PFNA dose corresponded to an internal dose of ~13-fold total human PFC exposure.

We did histology, hormone analysis, metabolomics in plasma and gene expression analysis in liver, testis and fat tissue. PFNA induced steatosis and general toxicity at the highest dose. At the lowest dose of PFNA, a clear potentiating effect of the cocktail on the PFNA-induced androgen levels was seen. Increases in dehydroepiandrosterone, androstenedione as well as testosterone were evident. On the other hand the cocktail protected against PFNA-induced increases in corticosterone levels. Gene expression analysis in low dose PFNA+Cocktail treated animals showed that CYP19 mRNA was affected in testis as well as in fatty tissues. A tendency towards increased UGTB15 and 17 mRNA in livers was observed indicating altered androgen metabolism. We suggest that metabolism of androgens is inhibited in livers and fatty tissues causing increased androgen levels. Thus, we conclude that interaction in the form of potentiation appeared in rats at doses close to human relevant exposure levels. The observations are effects at the molecular level, and it remains to be studied whether these effects translate into 'real' adverse effects.

Mixtures Research at the National Institute of Environmental Health Sciences

Cynthia RIDER

NIEHS/NTP, USA

BIOGRAPHY

Cynthia RIDER, PhD, is a toxicologist with the National Toxicology Program (NTP) at NIEHS, where she has been actively involved in developing mixtures research projects aimed at informing the cumulative risk assessment process. She serves as lead study scientist for a diverse portfolio of projects addressing the toxicity and/or carcinogenicity of test articles including polycyclic aromatic compounds and herbal products.

ABSTRACT

The mission of the National Institute of Environmental Health Sciences (NIEHS) is to discover how the environment affects people in order to promote healthier lives. Mixtures are ubiquitous in the environment, and the study of the environmental impact on human health necessarily involves mixtures. Consequently, NIEHS has engaged in diverse research initiatives to evaluate the toxicity of mixtures, ranging from epidemiological studies that intrinsically consider complex environmental exposures to toxicological studies of defined mixtures. Researchers in the Division of the National Toxicology Program (DNTP), the Division of Intramural Research (DIR) and the extramural community through the Division of Extramural Research and Training (DERT) have investigated a broad range of mixtures. Some examples of the types of mixtures studied include: groundwater contaminants, pesticides/fertilizers, dioxin-like chemicals (assessing the toxic equivalency approach), drug combinations, air pollution, metals, polycyclic aromatic compounds, technical mixtures (e.g. flame retardants, jet fuel), and mixed entities (e.g. herbal products, asbestos, nanomaterials). These evaluations have provided robust data on mixtures of interest. However, a coordinated mixtures research plan was not guiding previous NIEHS efforts. In 2011, an NIEHS workshop entitled "Advancing Research on Mixtures: New Perspectives and Approaches for Predicting Human Health Effects" brought together mixtures experts from risk assessment, exposure science, biology, epidemiology, and statistics to discuss major knowledge gaps in mixtures research and recommended approaches for moving forward. The main themes emerging from the workshop included: greater focus on complex exposures (through both monitoring and modeling), suggested tools for prioritizing mixtures for toxicological evaluation, application of systems biology approaches in mixtures research, critical evaluation of the utility of high-throughput platforms for evaluation of mixtures and chemical interactions, greater cross-disciplinary communication between toxicology and epidemiology, development of novel statistical methods for evaluating multiple pollutants in epidemiology studies, further development of approaches for determining sufficient similarity of complex mixtures, and the need for databases to house and integrate mixtures data across study types. Recently developed mixtures research projects at NIEHS have begun to address some of the identified knowledge gaps. Examples of ongoing projects include an evaluation of mixtures in the Tox21 high-throughput screening effort, a polycyclic aromatic compound mixtures testing program that incorporates sufficient similarity evaluation, and a project aimed at predicting interactions among mixture constituents based on a systems biology framework for identifying intersecting pathways of toxicity. Mixtures research continues to be a priority at NIEHS and future efforts will focus on addressing critical knowledge gaps and reducing uncertainties in assessing risk associated with exposure to mixtures.

SESSION 3
Risk assessment: case studies and methodologies

Grouping of pesticide active substances for cumulative risk assessment

Nathalie PRINTEMPS, Antony FASTIER
French Agency for Food, Environmental and Occupational Health & Safety (ANSES), France

BIOGRAPHY

Nathalie PRINTEMPS is a regulatory toxicologist working in the assessment of risks to human health of plant protection products and substances subject to REACh regulation. She also is involved in projects for the assessment of exposure to chemical mixtures and the development of the use of QSAR methods.

ABSTRACT

Regulation (EC) No.1107/2009 regarding marketing authorisation of plant protection products stipulates that plant protection product residues must have no hazardous effects on human health. Like regulation (EC) No.396/2005 regarding the maximum residue limits (MRLs) for pesticides in food or animal feed, it stipulates that the cumulative effects and/or the synergistic effects of pesticides must be accounted for in food risk assessments when the methods allow it.

Since 2007, the development of these methods has seen progress and their implementation will be based on the identification of the groups of active plant protection substances for which the assessment of cumulative effects has proved to be relevant. In other words, pesticides causing adverse effects on the same organs and/or with shared mechanisms of action can be included in a cumulative risk assessment.

In 2009, EFSA awarded a contract to the DTU (Technical University of Denmark) to conduct a study establishing a scientific status report with regard to the combined activity of chemicals in food and proposing a methodological approach to the assessment of cumulative risks. The report issued by the DTU in January 20122[1] shows that cumulative risk assessment methods, initially developed for mixtures of substances with similar effects, may also be suitable for combinations of substances with diverse effects. Knowledge of the target organs and the related mechanisms of action could make cumulative assessment possible.

ANSES (France) led a consortium composed of International Centre for Pesticides and Health Risk Prevention (Italy) and RIVM (the Netherlands) which was awarded a new EFSA tender to provide a complement to the information presented in the first report submitted by the DTU.

In this context, relevant data regarding the toxic effects of pesticides on the nervous system, on reproduction and development, and on the liver (including the biliary tract and the gallbladder) were collected and analysed. The data collected came from active plant protection substances approved as of 31 December 2011.

All approved plant production substances were reassessed with regard to these three categories. The analysis report showed that 244 substances affected the liver and/or the biliary tract, 67 substances impacted the nervous system, and 257 substances had an effect on reproduction and development.

[1]Danish Technical University (DTU), 2012. Identification of Cumulative Assessment Groups of Pesticides. External scientific report submitted to EFSA.

All the observed effects were listed substance by substance, indicating the No Observed Adverse Effect Level (NOAEL) or the Lowest Observed Adverse Effect Level (LOAEL). Certain identified or suspected mechanisms of action for these adverse effects were also mentioned.

The report, published by EFSA on 1 February 2013[2], provides an exhaustive database and an overview of the limitations observed during the collection of data used for setting up cumulative risk assessment groups (CAGs) for the systems under examination.

Cumulative Mixtures Risk Assessment: why simple formulas can work

Richard HERTZBERG

Emory University, USA

BIOGRAPHY

Richard HERTZBERG has a Ph.D. in Biomathematics, and specializes in quantitative risk methods for chemical mixtures. He teaches the graduate risk assessment course at Emory U, and researches risk frameworks and quantitative analysis of toxic interactions (multiple chemicals, and chemicals with nonchemical stressors). While at the U.S. EPA he led the risk assessment guidelines projects on chemical mixtures. He is a member of the Society for Risk Analysis and the American Statistical Association.

ABSTRACT

Estimation of health risks from exposure to multiple environmental chemicals (mixtures) is complicated by the lack of dose response information on the mixture being assessed, and the presence of additional stressors and population characteristics that can contribute directly or indirectly to the toxic effects of concern. While toxicology studies often seek understanding of the processes leading to adverse effects, risk assessment approaches seek to compile information that can help health officials make decisions about safety, intervention and clean up.

For chemical mixtures, a common risk assessment approach is to group chemicals by common toxicity and apply to that group either dose addition, or a modified dose addition that includes toxicological interactions. An understanding of the possible magnitude of toxic interactions is most important to risk assessors as an estimate of how much error would be in the assumed dose additive formula, which would be used for those many cases where full interaction information is lacking. Most numerical approaches do not work well for what the US EPA calls cumulative risk, i.e., the estimated response from combinations that also include nonchemical stressors such as psychological stress, socio-economic status, access to healthcare, and genetic predisposition. Examples will be presented of an interaction-based hazard index for chemical mixtures, a decision index for cumulative risk that incorporates environmental and vulnerability subindices, and a framework for uncertainty analysis of such approaches.

[2] ANSES/ICPS/RIVM, 2013. CFT/EFSA/PRAS/2012/07, 2013. Batch1 (RIVM), Batch2 (AOSACCO/ICPS), Batch3 (ANSES). Toxicological data analysis to support grouping of pesticide active substances for cumulative risk assessment of effects on liver, on the nervous system and on reproduction and development.

Effect-directed analysis (EDA) : a case study applied to endocrine disrupter identification in a river impacted by industrial discharges

Caroline GARDIA PAREGE[1], Marie-Hélène DEVIER[1], Nicolas CREUSOT[2], Selim AÏTÏT-AÏSSA[2], Hélène BUDZINSKI[1]

[1]Université Bordeaux 1, EPOC / LPTC – UMR 5805 CNRS, France - [2]Inéris, unité écotoxicologie in vitro et in vivo, France

BIOGRAPHY

Hélène BUDZINSKI is a CNRS research director in charge of the Laboratory of Physico- and Toxico-Chemistry of the environment a research group of EPOC (UMR 5805 University Bordeaux 1/CNRS). She is co-director of COTE LabEx. Her research focuses on various classes of organic contaminants (POPs, pesticides, pharmaceuticals, endocrine disruptors,...) studying presence, fate and toxic impacts. She has been involved in 10 ANR, in 7 European projects. She is the author 200 publications (h-index: 40).

ABSTRACT

A recent study reported the occurrence of strong reproductive alterations in fish from a French river. This observation could be related to the presence of an urban and pharmaceutical wastewater treatment plants (WWTPs). Indeed, wastewater effluents from urban and pharmaceutical factories are potential sources of endocrine disrupting compounds (EDCs) in aquatic ecosystems and these compounds can have adverse effects on the wildlife. In this study, passive sampling technique combined with *in vitro* mechanism-based bioassays was used to evaluate water contamination. In order to sample water while avoiding the daily fluctuation of contamination, polar organic compound integrative samplers (POCIS) were used.

Six monthly sampling campaigns at one upstream and two downstream sites of urban and pharmaceutical WWTPs were conducted.

Toxicological profiling of samples was evaluated using a battery of biosensors based on luciferase gene reporter (6 bioassays used) allowing the specific and integrative detection of a wide range of active chemicals. Strong activities were found in POCIS extracts with stronger activities downstream from the pharmaceutical factory. Targeted chemical analyses (LC-MS/MS) were performed on POCIS extracts and allowed the screening of 118 compounds (pharmaceuticals, antibiotics and steroids). 60 chemicals were found in this extracts but their contribution to the detected biological responses did not totally explain the activities.

In order to isolate the active chemicals and to identify them, effect-directed analysis (EDA) approach was used. EDA aims at the establishment of cause-effect relationships by sequential reduction of the complexity of environmental mixtures combining bioassays and fractionation procedures. Each fraction is subjected to various biotests (*in vitro* or *in vivo*) for the selection of active fractions.When the complexity of the mixture is sufficiently reduced, the ultimate active fractions are subjected to chemical identification (LC-MS/MS, LC-HRMS). In this study, POCIS crude extract was fractionated using Reverse Phase – High Performance Liquid Chromatography (RP-HPLC). Each fraction was then tested individually on *in vitro* cell lines. On the basis of RP-HPLC fractionation calibration, targeted chemical analyses were performed on several fractions. Steroid compounds like prednisolone, 6α-methyl-prednisolone, dexamethasone and

canrenone were detected in different fractions and these chemicals explained the majority of observed activities in the selected fractions. For many highly active fractions, compounds responsible for these activities remained unknown. To identify these active compounds a LC-HRMS system (LC-QTOF) was used. This procedure allowed the identification of several molecules among them drugs and steroids. As an example, the presence of a drug used for the treatement of amyotrophic lateral sclerosis, riluzole, was confirmed by these analyses in one of the most active fraction (activity remaining unexplained).
The activity of these identified chemicals remains to be confirmed on bioassays. This study highlights the usefulness of coupling EDA-based strategy with passive sampling technique to evaluate water quality and identify EDCs.

Keywords:
Wastewater effluents, polar organic compound integrative sampler (POCIS), effect-directed analysis (EDA), bioassay, RP-HPLC fractionation, non-target and target chemical analysis (LC-MS/MS, LC-QTOF)

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Cumulative risk assessment of 4 phtalates – A Danish experience

Rikke Donchil HOLMBERG

Environmental Protection Agency (EPA), Denmark

BIOGRAPHY

Rikke Donchil HOLMBERG is a trained biologist working with toxicology and human health risk assessment. Fields of work include chemicals management and hazard and risk assessment of chemicals, in particular of endocrine disruptors and combination effects of chemicals. She also works with international chemicals management, including the Strategic Approach to International Chemicals Management, SAICM and the Stockholm Convention on Persistant Organic Pollutants.

ABSTRACT

Chemicals are an integral part of modern life and serve a lot of good purposes. But, every day, we are exposed to a large number of chemicals from many different sources. During the last decade, increasingly convincing scientific findings have been published on the severe effects, combined exposures to chemicals, have on human health and environment. Our chemicals legislation is based on one-by-one assessments of single substances, not reflecting real world exposures.

Phthalates are a family of chemical substances based on the same general chemical structure, and are primarily used as plasticisers in PVC plastics.

Some phthalates are of human health concern due to effects on reproduction, and in the EU 5 low molecular weight phthalates are classified as toxic to reproduction, all considered to act via anti-androgen mode(s) of action.

The presentation will present and discuss the Danish work on and experiences gained from submitting a European restriction proposal on 4 phthalates based on cumulative risk assessment of DEHP, DBP, BBP and DIBP.

Case Study Application of the WHO Framework for Combined Exposures

M.E. (Bette) Meek

Institute of Population Health, University of Ottawa, Canada

BIOGRAPHY

Dr. Bette MEEK is currently the Associate Director of Chemical Risk Assessment at the McLaughlin Centre for Population Health Risk Assessment, University of Ottawa. She previously managed several chemical risk assessment programs within Health Canada.

With colleagues internationally, she has contributed to or led initiatives in areas such as weight of evidence analysis for mode of action, chemical specific adjustment factors, physiologically-based pharmacokinetic modeling, combined exposures and predictive modeling. She has authored over 175 publications in this area and received several awards for contribution in this domain. Dr. Bette MEEK has a background in toxicology receiving her M.Sc. in Toxicology (with distinction) from the University of Surrey and her Ph.D. in risk assessment from the University of Utrecht.

ABSTRACT

This presentation addresses the implications of recent advances internationally in the assessment of combined exposures to multiple chemicals, in an initiative led by the World Health Organization (WHO) Programme on Chemical Safety (PCS), involving collaboration with the Organization for Economic Cooperation and Development (OECD), the International Life Sciences Institute (ILSI) and the European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC). The WHO Framework for Combined Exposures is illustrated through consideration of case studies with objectives ranging from priority setting to full assessment.

Developments Illustrated through case study application include harmonized terminology and more efficient methodology for assessment, based on problem formulation and a framework which involves stepwise consideration of both exposure and hazard in several tiers of increasingly data-informed analyses

These developments build on experience in a range of national programs and incorporate predictive approaches in early tiers and increasingly refined, more data-informed and probabilistic analyses in later tiers.

Continuing and potential contribution of this experience internationally to evolving national programs is illustrated with emphasis on criteria for and potential contribution of predictive tools for grouping of chemicals, tailoring of effort to the appropriate level of complexity of assessment and the consideration and communication of sensitivity/uncertainty in tiered, increasingly refined approaches. Implications of this experience in considering priorities to increase the efficiency of risk assessment not only for combined exposures, but more generally are also addressed.

Mixtures and ways of regulating chemicals in the 20th and 21st centuries

Nathalie JAS

Unité RiTME – Inra, France

BIOGRAPHY

Nathalie JAS is an historian and sociologist of science and a researcher at the French National Institute for Agronomical Research (INRA). Her current work analyzes hazardous chemicals have been governed since the end of the nineteenth-century. She has recently co-edited two books on the topic, both with Soraya BOUDIA: *Toxicants, Health and Regulation since 1945* (Pickering & Chatto, London, 2013) and (*Powerless Science?: Science and Politics in a Toxic World* (Berghahn Books, Oxford & New York, 2014).

ABSTRACT

This paper puts the current mixture debate in a long term perspective. It shows that since the end of the 1940's chemical mixtures have regularly been (re)discussed by groups of scientists, worried by the possible detrimental effects of the presence in the environment of thousands of toxicology almost unknown chemicals. These discussions were held within the scientific frames of the period they took place, often integrating the newest chemical/biological knowledge of the time. Yet, until recently, these debates were never considered seriously by regulatory bodies. On the contrary the ways in which the political and administrative regulation of toxic chemicals had been developed de facto ignored them or set them aside.

The growing concerns over endocrine disruptors as well as the vivid socio-technic controversies they have given rise to, have again put forward the mixture issue, which this time has found its way within political debates and regulatory agencies. Because of the historical development of the toxicant regulation, the challenge faced by the regulatory bodies is now not only this of the scientific complexity of chemical mixtures effects, it is also this posed by regulatory paradigms and practices which are not designed for such issues.

Round table
How to address chemical mixtures in risk assessment? What are the challenges?

BIOGRAPHIES

Stefan SCHEUER is Director of his Brussels-based consultancy, specialised in EU environmental and energy policy and public affairs in support of public interests. His clients include a broad range of governmental, business and environmental organisations at EU and national level.Since 2011, he is appointed Secretary General of the Coalition for Energy Savings, the voice of European business and environmental associations, local authorities and trade unions for putting energy and efficiency at the centre of Europe's energy and economic policy. In 2007 Stefan Scheuer lectured EU environmental policies at the University of California and Edinburgh and carried out research on integrated water management.

From 2000 to 2007 he worked at the European Environmental Bureau, leading the Water Framework Directive and REACH campaigns, and steering the organisation's policy development as its Director. Stefan Scheuer holds an MSc in Hydrology, Albert-Ludwigs University in Freiburg, Germany.

Tim BOWMER joined ECHA in 2012 as Chairman of the Committee for Risk Assessment (RAC). He studied marine zoology at the National University of Ireland, Galway and was awarded a Ph.D. in 1982. Moving to the Netherlands in 1986, he worked for many years in the field of ecotoxicology and biodegradation testing of chemicals and later as a consultant on hazard evaluation and risk assessment. He has advised the UN International Maritime Organisation on the hazards of chemicals transport in bulk by sea since 1994 and has experience with many of the chemicals produced and transported as mixtures.

Tony MUSU gained a first degree in Chemical Engineering from the University of Brussels and a PhD from the Institut Pasteur in Paris. After his PhD, he spent 5 years as a researcher in industry. Since 2003, he has been working as researcher in the European Trade Union Institute's Health and Safety Department. On behalf of the European Trade Union Confederation, he takes part in various REACH-related EU working groups. He was Board member of the European Chemicals Agency (ECHA) in the period 2007-2011. He is also a member of the WG on Chemicals within the Luxembourg Advisory Committee on Health & Safety at work.

Bernadette OSSENDORP is a biochemist by training. She is Head of the Department Food Safety at the Centre for Nutrition, Prevention and Health Services of RIVM, the Dutch National Institute for Public Health and the Environment. She is member of the Joint FAO/WHO Meeting on Pesticide Residues (JMPR) since 2000, and a member of the EFSA Plant Protection Products and their Residues (PPR) Panel since 2006. She chairs the PPR Panel since 2012 and as such is now also a member of EFSA's Scientific Committee.

Cynthia RIDER, PhD, is a toxicologist with the National Toxicology Program (NTP) at NIEHS, where she has been actively involved in developing mixtures research projects aimed at informing the cumulative risk assessment process. She serves as lead study scientist for a diverse portfolio of projects addressing the toxicity and/or carcinogenicity of test articles including polycyclic aromatic compounds and herbal products.

Professor Dr. Gilbert SCHÖNFELDER heads the Experimental Toxicology Department and ZEBET at the Federal Institute for Risk Assessment (BfR). Gilbert SCHÖNFELDER is a well-established expert, provider of expert scientific opinions and renowned scientist in the area of Pharmacology and Toxicology. His research focuses on experimental toxicology, the development and validation of alternative methods to animal experiments and reproductive and developmental toxicology. He is the Chairperson of the Hormonal Toxicology Section of the German Endocrinology Society and pharmacological and toxicological expert for national and international institutions, committees and law courts. In 2008, he was appointed expert to the BfR Committee for Contaminants and other Undesirable Substances in the Food Chain. He was provider of expert opinions within working groups of the World Health Organisation (WHO).

The focus of his work for the BfR will be the molecular and experimental toxicology. Schönfelder's goal is to unravel molecular mechanisms of the effects, at the organ and cellular level, of chemicals in their conventional form and also in nano size. This knowledge is the basis for the development and validation of methods that are to replace the legally required animal experiments in the safety assessment of chemicals.

Lisette VAN VLIET is Senior Policy Advisor on Chemicals and Chronic Disease Prevention for Health and Environment Alliance (HEAL) in Brussels. She holds a Ph.D in international relations and environmental studies from the Australian National University in Canberra. Lisette represents HEAL on REACH and other EU policies on harmful chemicals such as endocrine disruptors. She conducts outreach to groups on the prevention of chronic diseases through environmental policy measures, and represents HEAL member and partner expertise to the EU institutions.

Marco VIGHI, Professor of Applied Ecology and Ecotoxicology at the University of Milano-Bicocca. Main research fields: environmental toxicology of micro pollutants on aquatic and terrestrial ecosystems; QSARs for organic chemicals; environmental distribution and fate of contaminants; long range transport of POPs; effects and fate of mixtures; ecological risk assessment. Author of 160 scientific papers and books. Member of Scientific Committees on Ecotoxicology of the European Commission from 1991 to 2013.