

Conference Reports

International Workshop

Internal Exposure – Linking Bioavailability to Effects

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In the workshop 'Internal Exposure', which took place from August 22 to 27 in Monte Verità, Switzerland, scientists from the fields of ecotoxicology, environmental chemistry, environmental and human health risk assessment, toxicology, and biopharmacy were brought together. The objectives were (i) to discuss recent developments and insights in (internal) exposure measurement and modeling in each of these disciplines and (ii) to stimulate scientific exchange and research collaborations among these fields. A particular focus was on tools and models to relate external concentrations to internal distribution in organisms and to relate internal concentrations to mechanisms and effects.

Quantification of toxicity of a given chemical or mixture of chemicals is commonly based on external exposure measurements. However, for the understanding and interpretation of pharmacological and toxicological effects, the exposure within an organism or even at the target site is a much more suitable parameter than the external exposure concentration or dose. Understanding the route from external to internal exposure is relevant in several research areas and scientific disciplines. The concept of internal effect concentrations has been recognized in ecotoxicology in the late 1980s as a useful concept to relate bioaccumulation to effects and has also been advocated to be useful for environmental risk assessment. However, only few examples have been published and the concept has not yet found its way into regulation.

While research within the environmental sciences on the issue of internal exposure and modes of toxic action is only starting, more advanced physiologically-based toxicokinetic or pharmacokinetic models have been introduced in human health risk assessment and pharmaceutical sciences to relate external exposure to internal exposure and target exposure. In this workshop, the state of the art in this research field was evaluated from the different perspectives of environmental and health sciences. The overall theme was divided into four subtopics, covering all steps on the way from exposure to effect: (1) Bioavailability and Partitioning, (2) Uptake and Toxicokinetic Processes, (3) Linking Exposure to Effects, and (4) Integrative Approaches in Risk Assessment.

70 scientists from Europe, North America and Australia participated in the workshop. There was a good mix between established researchers and young Ph.D. students and postdocs, between researchers, consultants, industry and agency employees, as well as between more environmentally and more health oriented participants. Almost everybody had one or multiple tasks, as lecturer, poster presenter, discussion leader, reporter of discussion groups, etc. and everyone participated actively. The workshop was characterized by a friendly and open atmosphere. Discussions were intensive and very productive. New research

collaborations were initiated and the understanding between health and environmentally oriented researchers, who rarely meet, was improved. There was a consensus that the workshop should be followed up by a second meeting in two to five years.

Lectures

In the first session, entitled **Bioavailability and Partitioning**, concepts of bioavailability of organic and metallic pollutants were covered. DOMINIC DiTORO from the University of Delaware, U.S.A. set the stage with the opening lecture on understanding bioavailability and mechanisms of toxicity and how these issues impact risk assessment and the development of quality criteria. His plea for understanding the science behind, but developing and using simple models, was taken up again and again throughout the workshop. CHRIS ROTH from EAWAG in Switzerland advocated the use of polyparameter, linear, free-energy relationships for describing and understanding sorption processes in the environment, including biological systems, and as a diagnostic tool for understanding species sensitivity differences in toxicity. JUSSI KUKKONEN from Joensuu University in Finland illustrated how sediment characteristics affect bioavailability of organic contaminants. Biomimetic extraction techniques are a particularly promising approach for investigating free and bioavailable concentrations. MINNE HERINGA from KIWA in the Netherlands showed examples on how these techniques may improve the estimation of target doses in *in-vitro* assays. MICHAEL MCLACHLAN from Stockholm University in Sweden presented models that link environmental concentrations to human tissue levels. Finally, STEVE MAUND from Syngenta, Switzerland, closed the session with examples of practical applications in the risk assessment of pesticides, and pointed out that internal exposure could be a tool for integrating time-variable exposure, but that there is still a long way to practical implementation.

The next topic on the way from external to internal exposure was **Uptake and Toxicokinetic Processes**. Here, the focus was on mechanistic aspects of bioaccumulation and/or toxicokinetic models, including issues of metabolic activation and deactivation. An attempt was made to integrate knowledge from health sciences into environmental science and to extend the topic from classical models of humans and mammals to models of environmentally relevant species, e.g. members of the aquatic food chain. RAYMOND YANG from Colorado State University, U.S.A., illustrated the application of physiologically-based pharmacokinetic (PBPK) and reaction network modeling in human health risk assessment as a potential tool to cope with cumulative exposure and exposure to mixtures of chemicals and stressors. To calibrate those models, a fairly good understanding of the transport properties of chemicals in an organism is needed. ALEX AVDEF from Pion, U.S.A. showed how permeability and reten-

tion of pharmaceuticals and environmental contaminants in biological membranes are experimentally determined. A further application of PBPK models is to link in-vitro and in-vivo studies as was convincingly demonstrated by ANDREAS FREIDIG from TNO, the Netherlands. PETER LANDRUM from the Great Lakes Environmental Research Laboratory, U.S.A. extended the kinetic approach by considering both toxicokinetics and toxicodynamics in an attempt to model time dependence of toxicity and mixture effects in aquatic organisms.

The third topic was **Linking Exposure to Effects**. STEVE BRADBURY from the U.S. EPA showed how hypothesis-driven approaches to establishing information needs may optimize resources, cost and time in generating data for risk assessment. He emphasized the particular role of toxicity pathways and modes of toxic action as low-tier decision criteria. RIK EGGEN from EAWAG, Switzerland, and KRISTIN SCHIRMER from UFZ Leipzig, Germany, introduced molecular biology and cell-based in-vitro systems as potential tools for a mode-of-action-based effect assessment. The step from the laboratory scale to the field scale was illustrated by JOCHEN MÜLLER and RENÉE MULLER from Queensland University, Australia, with an example of a site-specific risk assessment in surface waters using a combination of passive sampling technology with an ecotoxicological test battery.

The fourth and last topic, **Integrative Approaches in Risk Assessment**, integrated the topics discussed during the first three days. JOHN GROTEN from TNO, the Netherlands presented approaches for human health risk assessment of chemical mixtures. TOM PARKERTON from Exxon Mobil Biomedical Sciences, U.S.A. showed how a combination of biomimetic extractions and mixture toxicity concepts provide a tool for assessing the toxicity of complex hydrocarbon mixtures to aquatic life. GERRIT SCHÜRMANN from UFZ Leipzig, Germany, employed Qualitative and Quantitative Structure Activity Relationships not only for prediction of toxicity, but also for classification of modes of toxic action. Finally, GLENN SUTER from U.S. EPA proposed an approach how internal exposure could link health and ecological risk assessment and explored commonalities of both fields. His final conclusions to use multiple approaches including external, internal and site-of-action concentrations as a weight-of-evidence approach to assessment was taken up as one of the important conclusions of the workshop.

Poster Sessions

More than 40 posters were presented covering the four topics introduced above. Poster sessions proceeded in a very stimulating atmosphere. A poster award committee selected three posters for oral presentations. JAY GAN from the University of California, Riverside, U.S.A. with his study on the bioavailability of synthetic pyrethroids in surface waters using SPME technology won a one-year subscription of Environmental Science and Technology. THOMAS TER LAAK from Utrecht University received a copy of the textbook Environmental Organic Chemistry for his poster on a solid phase dosing and sampling technique for very hydrophobic compounds in complex matrices. SIBYLLE KAISER from EAWAG received a one-year subscription of Environmental Science and Pollution Research for her study on the risk assessment of complexes of hydrophobic ionogenic organic compounds with metals.

Discussion Groups

One crucial asset of the workshop was the discussion in smaller break-out groups. Five groups of 10 to 16 participants met

twice and presented their results to the plenum in the closing session on Thursday night. In the following, there is a brief summary of the discussions provided by the discussion leaders:

1. New developments and concepts in bioavailability measurements and biomimetic extractions

Discussion leader: Heather Leslie

Bioavailability refines our understanding of exposure to environmental contaminants in air, water, sediment and food. When considering internal exposure, there is common ground in the bioavailability issues for both human toxicology and ecotoxicology – it is ultimately the dose at the target site that determines the effect, and the common goal is to come to understand the bioavailability and partitioning processes that contribute to the dose reaching the target.

When does bioavailability really count? In the environment, bioavailability is important to take into consideration when adverse effects are observed while exposure is significantly limited by bioavailability and when prioritizing sites for remediation. In humans, bioavailability is similarly important in risk assessments when the chemical's properties enables it to be taken up from a given source and effectively reach target sites.

The pragmatic approach to defining bioavailability in this discussion was to specifically state for each case how the measurement is made and which parameter is being measured. After all, bioavailability measurements are all approximations of one sort or the other – precise measurement of the genuine bioavailability of a chemical to a particular organism at a particular time and place may be too tall an order to fill anyway.

How can bioavailability be studied? A long list was made of methods currently being used and under development for assessment of the bioavailable fractions and potential bioaccumulation by chemical extractions.

Determining internal residues in biota (e.g. fish, pine needles) gives a direct indication of bioavailability, but also integrates many other species-specific and other modifying factors, such as metabolism, microhabitat, feeding behavior, etc. However, there are major drawbacks for using measured residues in biota on a regular basis, including time and budget-consuming procedures and the fact that it is inconvenient for risk assessment standardization due to high variability in biotic sample concentrations.

Regulators cannot make use of bioavailability measurements unless they are robust, repeatable, reproducible, cover a large spectrum of graded response and, last but not least, relevant for the parameter of interest. The lack of quality control has been somewhat of an obstacle for the widespread use and implementation of bioavailability measurements of organic chemical contaminants to date.

The future of bioavailability measurements holds requirements for more standardization, validation, automation, development of fast and low cost analyses, and for increasing the number of chemical classes that can be readily assessed with the existing methods to include, for example, ionizing chemicals and polar functional groups. Furthermore, this may involve developing tools with new materials (e.g. gels, resins, membranes). Surrogate phase testing for biomimetic suitability can be aided by polyparameter LFERs, making it easier to make predictions about the behavior of different chemical classes.

Any discussion of bioavailability measurements is complex. Besides scientific details it encompasses the requirements of regu-

lators and risk assessors for robust, cost-beneficial measurements, the integration of thinking both 'eco' and 'human', communicating across fields of study, and bringing theory into practice in real world investigations and problem-solving.

2. In vitro-in vivo extrapolations

Discussion leader: Pat Schmieder

The group emphasized that when using in vitro systems for study of toxicodynamics it is important to also characterize chemical kinetics within the system to the extent possible. Combined kinetic and effects' information is key to establishing linkages across levels of biological organization, with greater understanding of toxicological processes gained at lower levels of organization, but increased relevance to the in vivo state gained from assays representative of higher levels of organization.

Significant expectations that have been placed upon the development and potential use of in vitro assays to reduce, refine and eventually replace in vivo testing was the subject of much discussion. It was acknowledged that, while research and risk assessment scientists are charged with developing and applying in vitro systems, assessing what toxicological understanding is gained from an in vitro test system, and determining the relevance to an in vivo situation, it is the risk managers who play a key role in identifying goals and acceptance criteria for the ultimate use of such information. Additionally, there are many external stakeholders that have high expectations for the use of in vitro data; expectations that may or may not be aligned with the current state-of-the-science. In general, it was felt that there are current examples of in vitro assays that can and have been used to reduce the number of in vivo tests needed to yield adequate information to accomplish risk management goals. In vitro tests have also found application in refining in vivo test designs by allowing focus on the most critical aspects of the kinetic and/or toxicological response needing measurement, thus allowing reductions in animal usage and resource commitments. However, while it was recognized that there are significant legislative and social concerns striving for complete replacement of animal tests, there were no current examples of full replacement of in vivo with in vitro testing identified.

Currently, some of the best uses of in vitro data are: 1) to gain mechanistic understanding of toxicokinetic, metabolic, or toxicodynamic processes yielding information complementary to in vivo data, but not, as yet, an absolute replacement for in vivo; and 2) to predict the potential for chemical action: for chemical hazard identification, to prioritize chemicals for further screening/testing, to contribute to the weight-of-evidence when interpreting in vivo toxicological data. Of the few in vitro assays known to have gained widespread regulatory acceptance as indicators for potential adverse effects (e.g. Ames test to indicate mutagenic potential), they are accepted because they have been applied broadly enough to know their advantages and limitations. It therefore seems the responsibility of the developers and/or proponents of any new in vitro assay intended for a particular use to list not only the advantages of the system and what information it provides, but also to discuss any known limitations of the assay.

The group felt it is very important to state the intended use of an in vitro assay. As we seek to gain acceptance of in vitro models for a variety of applications, the question invariably arises as to how well information from the in vitro model can be extrapolated to predict something about in vivo activity. In many situations assays are used for a purpose different than originally proposed and evaluated. While assays based upon the same basic principle may be modified to meet a variety of needs, each variation must be evaluated for how well it is suited for the intended use. Additionally, in vitro assay data could be used to prioritize chemicals for more integrative testing if it has been demonstrated that a measured in vitro activity is plausibly linked to a whole organism effect of risk assessment concern. A plausible linkage between in vitro measures and in vivo effects can be established by using more than one in vitro assay representative of different levels of biological organization along a defined pathway of toxicity. To determine if the degree of uncertainty is acceptable for the stated use, it is advantageous to define where the linkages are most certain or most variable.

3. Application of PBPK modeling to cumulative risk assessment

Discussion leader: Raymond Yang, Reporting: Kathrin Fenner

In 1996, the Food Quality Protection Act (FQPA) in the U.S. provided the necessary impetus for the USEPA to consider cumulative risk assessment. In doing so, the USEPA deviates from the traditional single chemical risk assessment approach by considering multiple chemicals, multiple uptake pathways and multiple possible exposure media. The Office of Pesticide Programs (OPP), USEPA, took the lead and conducted cumulative risk assessment on organophosphorus (OP) pesticides. More recently, the Office of Prevention, Pesticides, and Toxic Substances (OPPTS) and the National Center for Environmental Assessment (NCEA), USEPA, develop the framework of incorporating physiologically-based pharmacokinetic (PBPK) modeling into the cumulative risk assessment process. In this Workshop, therefore, we formulated a Working Group to specifically discuss the pros and cons of the application of PBPK modeling to cumulative risk assessment. The Working Group had two very active discussion sessions. A brief summary is given below.

The current default assumption for assessing the mixture toxicity is based on the additivity concept for chemicals with a common mode of toxicity. However, opportunities for non-additive behavior, i.e. synergistic or antagonistic interactions, may occur both in the pharmacokinetic or pharmacodynamic (PD) phase. The following possible mechanisms of non-additivity were suggested: (i) Interference of one or several compounds with metabolism or cell regulation processes; (ii) Saturation of the target enzyme site; (iii) Compounds exerting more than one common mechanism of toxicity. Although PBPK modeling will be useful, the processes identified above are not commonly accounted for in most present-day PBPK models. Thus, in Table 1, suggested further developments of PBPK models are listed in order for them to be suited to represent various types of non-additive behavior.

Table 1: PBPK models

Mechanism of non-additivity	Necessary amendments
Interference with metabolism	Inclusion of metabolic pathways and explicit modeling of pharmacokinetics for reactive metabolite(s)
Interference with cell regulation	Modeling of interactions between chemicals and cell regulation networks
Saturation of target enzyme site	Explicit modeling of PD processes → PBPK/PD models
Several mechanisms of toxicity	Development of related PBPK/PD models

With further development, PBPK/PD models can be promising tools for modeling non-additive behavior of mixtures. More critical in the entire process is the clarification of the actual mechanisms that lead to non-additive behavior in a given system. Here, the amended PBPK models could be of particular value, not being as predictive, but as hypothesis-generating tools that help to guide experimental work on non-additive effects. Further down the road, the linkage of multimedia fate and multipathway uptake models with multi-substance PBPK/PD models was identified as a goal to strive for.

4. How to deal with multiple uptake routes and mixtures in the regulatory setting?

Discussion leader: Joop Hermens, **Reporting:** Rolf Altenburger

With regard to the assessment of mixtures of contaminants, it might be productive to distinguish between different problems like complex, undefined exposure situations versus defined multiple substance exposure; and also between various scopes for assessment like predictive product or process assessments versus retrospective site-specific or spill-related exercises. In this respect, combined effect assessment can only be as good as the exposure analysis. The theoretical basis of generating non-interaction expectations for a biological response for a contaminant mixture from the knowledge of the effects of the individual components is regarded as well-established. While concentration addition seems to work for substances in line with the simplistic pharmacodynamic assumption of a similar mode of action, the alternative concept of response addition provides a reasonable non-interaction model for dissimilarly acting components. An array of tools including measuring techniques (WET, TIE, bioassay-directed analysis) and models (risk estimates, effect indices, parameterized responses, response surfaces) for forensic and predictive approaches are established and might be further developed. There is a prospect that novel techniques related to -omics techniques (genomics, proteomics and metabonomics) might in future help to bring more light into black box modeling of organism responses or help to understand cases of interaction. Regarding the transfer of current knowledge into regulation, it was felt that this is still an issue as the rules for coping with combined effects in a standard setting are not consistently worked out (e.g. the understanding and requirements of mode of action information). Research was felt necessary with respect to extending current knowledge towards conceptually thinking, including interaction between chemical contamination and other stressors (e.g. UV, pathogens). Furthermore, the relevance of biological qualities at higher organizational levels (e.g. differences in species sensitivity) for the prediction of combined effects is not understood. Thus, as we go from single species evidence into ecosystems, the chance that our extrapolations fail increases.

Identified challenges include:

- The analysis of differential fate and transport properties of complex mixtures
- The operationalization of mode of action and multiple mode of action concepts
- The analysis of combined effects at chronic levels (reproduction, development).
- The merge of conceptual prospective (inductive) knowledge and diagnostic (deductive) approaches in the assessment of actual mixture problems.
- The modeling of pulsed (or sequential) exposure situations

5. How can internal exposure link human health and environmental risk assessment?

Discussion leader: Monika Nendza,

Reporting: Matthew MacLeod

A working group was formed to discuss how the analysis of internal exposure data could help link human health and environmental risk assessment. The group quickly broadened the scope of their task to address the more general question of how to develop methods that allow toxicological information to be extrapolated across taxonomic groups.

A need exists to extrapolate information between species because of insufficient data for most species, and because of a desire to limit costs or animal testing and to make the most efficient use of available data. Attempting these inter-species extrapolations allows regulatory decisions to be made based on a 'weight of evidence' approach.

Endpoints that are relevant across a broad range of taxonomic groups were identified, for example mortality, growth, mutagenicity, and developmental and reproductive impairments. It was agreed that to be most scientifically defensible, extrapolations should be made across as narrow a range of taxonomic distance as possible, however, this could be overruled in policy making by a desire for precaution. However, the group agreed that extrapolations could only be made across a high degree of taxonomic distance if high uncertainties were acceptable.

Several methods that represent different degrees of mechanistic understanding were identified for making interspecies links between data. For example, statistical models, allometric relationship, models to predict biotransformation rates, and physiologically based pharmacokinetic (PBPK) models that are particularly powerful when used in conjunction with the critical toxic residue approach. Many of these methods are in current use (Table 2).

Table 2: Examples of currently applied methods for extrapolation of toxicological data across species

In-vitro screening tests, e.g. mutagenicity tests to screen for carcinogenicity
Species sensitivity distributions
Regression equations
Allometric models

The combination of PBPK models with information on chemical concentrations at the site of action that induce effects were identified as a particularly promising method for providing policy makers with more powerful tools for making inter-species connections. More research is required to improve extrapolation methods for toxicokinetics, particularly estimation of biotransformation rates and pathways, but also processes such as absorption and excretion, and also for toxicodynamics, including improving effect assessment according to mechanism and site of toxic action, and organism repair mechanisms. There is a need to identify cases where extrapolations can be made at different levels of biological integration, i.e. at the cellular, organism and even population-level. A major emphasis should be placed on development of quantitative methods to estimate uncertainties associated with extrapolations across taxonomic groups.