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Overview of an International Workshop on In Vivo Fish Bioaccumulation Databases

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Assessing the bioaccumulation (B) potential of chemicals is an important current and future regulatory consideration in chemical management policies across the globe. A dataset of reliable in vivo B data is needed as a reference to support consistent regulatory assessments of specific chemicals, to serve as the basis for developing and/or validating models and alternative methods that are faster, cheaper and avoid animal use, and to give insights into inter-species and lab-field extrapolation of B data. To progress the development of a B in vivo database, a workshop sponsored by ILSI-HESI and involving B experts from industry, academia, and government, was conducted in November 2005. Workshop participants reviewed the availability and content of existing B databases worldwide, developed guidance for study quality criteria that should be considered when conducting B tests or judging their reliability, produced recommendations ! for the OECD on their BCF data template, and proposed steps to implement improved data sharing across government databases owners and modelers.

Overview of a Workshop Examining the Use and Development of *In vitro* Techniques for the Assessment of Bioaccumulation in Fish

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The bioaccumulation (B) of a chemical can be evaluated by exposing fish via water or food. Such an OECD 305 test can exceed $100,000, due to the need to provide consistent exposures, measure chemical concentrations, and observe >100 fish. The paucity of *in vivo* data on the large universe of chemicals that are lipophilic is driving development of reliable lower-cost approaches. Several approaches involve *in vitro* techniques to estimate the absorption, distribution, metabolism and excretion (ADME) of chemicals which are used to predict chemical behaviors that impact B. To progress the development of *in vitro* techniques for application to B assessments, a workshop sponsored by ILSI-HESI was conducted in March 2006. This presentation reviews the workshop presentations and guidance on the development and application of *in vitro* techniques for the assessment of bioaccumulation. Participants were from academia, industry, government research and

regulatory, representing a range of global perspectives. Over the course of two days, workshop participants reviewed existing *in vitro* techniques for mammals and fish, identified gaps in techniques and applications, and developed guidance to address gaps.

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Lessons learned from the Evaluation of Bioconcentration and Bioaccumulation Factors of Commercial Chemicals

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The recently ratified UNEP protocol on Long Range Transport of Atmospheric Pollutants is causing various countries around the world to evaluate, categorize and prioritize thousands of commercial substances for their persistence, ability to bioaccumulate and toxicity. To assess the bioaccumulative properties, most jurisdictions make use of the octanol-water partition coefficient (Kow), the bioconcentration factor (BCF) and the bioaccumulation factor (BAF) and apply comparable criteria. This poster presentation summarizes a set of lessons learned from the evaluation of 5,317 bioconcentration factor (BCF) and 1,656 bioaccumulation factor (BAF) values in 219 aquatic species for 842 organic chemicals from 392 scientific literature and public database sources. The analysis indicates that there are empirical data available for less 3.7% of the chemicals in commerce in Canada, illustrating the importance of the selection of appropriate criteria and methods to evaluate the bioaccumulation behavior of the remaining chemicals. This poster summarizes several lessons we learned from the evaluation of bioaccumulation data and proposes a set of recommendations with regards to definitions, objectives, criteria values, data quality criteria, alternative experimental methods and the application of models and computational techniques that may be useful to regulators, industry and scientists interested in bioaccumulation.

Relevance of free concentration measurements in bioaccumulation and toxicity studies

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Concentration measurements form the basis for the generation of numerous experimental parameters and many decisions are based on reported measured concentrations. Bioconcentration factors of highly hydrophobic compounds for example use aqueous

concentrations in the denominator, although it is known that concentration measurements in aqueous solutions of hydrophobic chemicals are extremely difficult to perform and are often subject to systematic errors. Total concentrations in soil and sediment are still often used by regulatory agencies in risk assessment decisions, while differences in bioavailability may strongly affect a site-specific risk. Also in vitro systems often report a dose or a concentration at a certain biological effect. However, as the precise exposure in these systems is often not investigated, such data and also the conclusions based on these data are often highly uncertain. This is one of the reasons why quantitative in vitro assays are poor in predicting quantitative in vivo effects. In this presentation we advocate the use of measured freely dissolved concentrations as a more intrinsic concentration parameter. Experimental data show that bioconcentration factors in soil and sediment organisms, calculated via freely dissolved concentration, do not show the often observed non-linearity with octanol-water partition coefficients. We will show that effect concentrations based on freely dissolved concentrations represent the more intrinsic potency of chemicals in in vitro assays. These data provide unbiased input for computational methods, and can shed an entirely different light on the activity of chemicals. Finally, examples will be presented on measured free concentrations in relation to bioavailability in soil. Besides the focus on examples, also techniques for measuring freely dissolved concentrations will be briefly discussed with an emphasis on solid phase micro-extraction

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Bioconcentration of primary amines in fish – too B or not too B?

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Key words: Cationic surfactant, adsorption, bioaccumulation, bioconcentration

Hexadecylamine (C16PA) is a component of a group of cationic surfactants currently under scrutiny in the EU existing substances (793/93) risk assessment process. Technical problems are associated with measuring the lipophilicity and bioaccumulation potential of these molecules. Laboratory measurements and off-the-shelf models do not provide accurate values of log Kow and no bioconcentration data exist. A modified OECD 305 bioconcentration test, reducing the number of fish, was performed to determine bioconcentration of C16PA in *Cyprinus carpio*. Plateau levels of C16PA were reached

within days of exposure but much C16PA was ionically or hydrophobically bound to the outer surfaces of the fish, notably to the mucous and scales while considerably less was within the fish tissues, including the gills. Remaining work was dedicated to the quantification of the internal and external fractions of C16PA to estimate the bioconcentration factor. Problems and techniques used to solve them are outlined.

Tiered approaches in silico assessment of bioaccumulation

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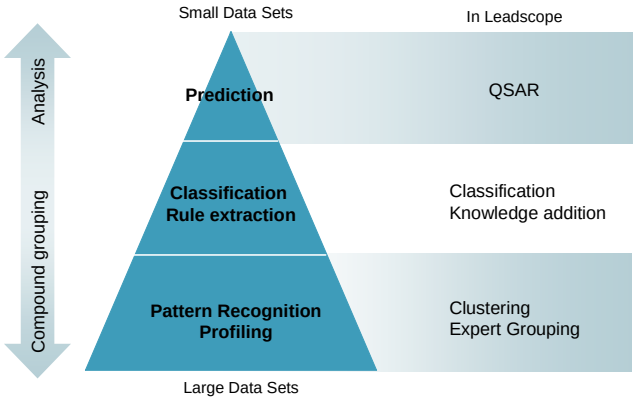
The assessment of the fate of a chemical as an environmental stressor requires a very complex process based on classifications of compound potentials in Persistence and Bioaccumulation. Due to the limited size and quality of the data from conventional testing, which often relies on animal testing, improved methods are being developed in relation with EU policies such as REACH and 7th Amendment to the Cosmetics Directives. A rough binary schematic diagram of a tiered approach can be devised for PB classifications. Further, the subsets of compounds within each tier can be quantitatively modeled. For quantitative modeling, metabolism is one of the most important factors in a chemical's ability to bio-accumulate. Any models relating $LogK_{OW}$ to LogBCF without reflecting the metabolic fate need to be improved. In this poster, a flexible data mining system that allows the user to add metabolic rules is demonstrated. By clustering the datasets for structures, two compound classes are compared for building models with and without the metabolic knowledge addition. For example, the biphenyl polychloride class modeled well without metabolic rules, while modeling the halophenols class was substantially improved by adding metabolic rules. Therefore, a predictive data mining system, where metabolic reactivity knowledge can be added on-demand, will greatly assess this tiered approach of bioaccumulation.



A Pragmatic Approach to Reduce the Workload in B Assessments

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Key words: B Cutoff value based on Kow, REACH PBT & vPvB Assessment

Article 13 of the REACH legislation (Council Draft December 2005) requires notifiers to carry out a PBT & vPvB Assessment. As REACH covers a huge amount of substances it is crucial for notifiers to have assessment guidance available preventing that substances have to be assessed on B which are in fact not bioaccumulative. In the EU the B Criteria is related to BCF (e.g. 2000 for PBT or 5000 for vPvB substances). As for very many substances BCFs are not available Kow is used as B criteria (potential B with Kow > 4.5 for PBT or > 5 for vPvB Substances). From available data is known that at higher Kow bioconcentration, bioaccumulation and biomagnification drops again. In the framework of the EU REACH Implementation Project RIP 3.2-2 'Commission Working Group on PBT & vPvB Guidance' a first draft proposal was delevoped for a Cutoff for B Assessment for substances with calculated Kow > 9.5. In addition if a substance fulfills this cutoff criteria it has to be checked if potential metabolites are not PBT or vPvB substances. This approach was tested with five substances from the EU PBT & vPvB List (originally 93 substances) and was found applicable. Another 10 substances from this list with calculated Kow > 9.5 are currently under investigation to check applicability.

Use of a Parallel Artificial Membrane System to Evaluate Passive Absorption and Elimination in Small Fish

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Evaluating bioconcentration has been of critical issue for decades. There are needs for developing alternative screening methods in a tiered approach due to the large number of synthetic chemicals that need to be evaluated and high cost of existing test methods using aquatic animals. For this reason, a parallel artificial lipid membrane permeability assay (PAMPA) that is a well established tool in pharmacokinetic research was explored and evaluated for its potential to mimic passive mass transfer of hydrophobic organic chemicals in fish. In this model system, a membrane filter-supported lipid bilayer separates two aqueous phases that represent the external and internal aqueous environments of fish. To predict bioconcentration kinetics in small fish using this system, literature absorption and elimination rates were analyzed using an allometric diffusion model to quantify the mass transfer resistances in the aqueous and lipid phases of fish. The impact of the aqueous phase mass transfer resistance was controlled by adjusting stirring intensity to mimic bioconcentration rates in small fish. Twenty three simple

aromatic hydrocarbons were chosen as model compounds the evaluation. For most of the selected chemicals, literature absorption/elimination rates fall into the range predicted from measured membrane permeabilities and elimination rates of the selected chemicals determined using the diffusion model system. After further refinement, this in-vitro system may be a valuable tool for bioconcentration assessment and prioritization of bioaccumulation testing.