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Decision Tree for Evaluating Bioaccumulation Potential in Fish

Introduction

Due to the limitations of computer models and *in vivo* fish bioconcentration data, a cross-sector HESI SETAC Working Group (March 4, 2006) was requested to design a decision tree that could be used to improve how the potential for bioaccumulation ("B") of chemicals is assessed. The aim of the decision tree is to indicate how additional information about various properties of a chemical could be incorporated into current B assessment methods, and by doing so, improve both the understanding of a chemical's environmental risk and categorization decisions for PBT programs. Further, this effort could help to focus and prioritize research and funding on what will most greatly influence the decision-making process concerning materials that have a potential for "B" in the environment.

The "Decision Tree" provides a step-by-step guide to decisions on the next steps to take when determining the potential for "B" for any material.

The specific goals are to:

1) Maximize our understanding of bioaccumulation potential while minimizing the use of animal testing.
2) Minimize testing requirements through the use physicochemical data and structure-activity relationships (SAR).
3) Reduce the time needed for xenobiotics to be evaluated.
4) Improve prioritization and identification of chemicals for further study.
5) Increase understanding of SAR.

To accomplish these goals, "B" assessment is done in a tiered fashion. As described here:

Tier 1 – Initial Screen for "B"

Within the regulatory arenas where "B" categorization is mandated, they are deciding on a process that could include basic empirical bioconcentration data as well as computer models for estimating "B" potential. The results of these models or data are then compared to the pre-determined criteria for "B". If the chemical is determined to be "B" based on this approach, the chemical should pass to further evaluation in the subsequent tiers of the "Overall "B" Decision Tree (Figure 1).

Tier 2 – Paper Screening Exercise

The first step in the subsequent evaluation process is to gather all the data on the chemical and conduct two types of paper screenings. The first screening is to determine if the substance is present in the aquatic environment and then if the substance is likely to remain in the aquatic environment; both indicate whether aquatic organisms can be exposed. Multi-compartment fate models, such as a Level III fugacity model, can provide a useful screen of the potential of a compound to occur in various compartments in the environment, including the aquatic environment. If this first screening identifies that organisms are unlikely to be exposed, further tiers of assessment are unnecessary. However, if exposure is possible, a second

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screening is conducted to determine if absorption across biological membranes is impeded (e.g., chemical is not bioavailable, too large, etc.). If absorption is possible, do Tier 3.

Tier 3- Absorption potential

This part of the decision tree aims to provide estimates on two processes that govern the absorption potential of chemicals:

- Environmental availability of the compound for absorption by the organism.
- Ability of the compound to cross the biological membranes/epithelia to enter the organism.

Considering the currently available tools, a three-pronged approach appears feasible:

- Physicochemical parameters to provide baseline information (i.e., "Lipinski's Rule of Five" adapted for use with fish).
- Biomimetics or passive samplers such as SPMD, SPME, EVA.
- Biological models.

If a chemical appears to be bioavailable and absorbed, its metabolic lability is then considered.

Tier 4 – Metabolism Assessment
(Figure 2)

The first step would be to use a simple *in vitro* screen for metabolic potential (e.g., in S9 liver fractions or hepatocytes). If no significant biotransformation of the compound is observed or greater certainty is needed, then some type of *in vivo* study (e.g., cannulated fish) or *ex vivo* study (e.g., liver perfusion) could be initiated to estimate the impact of low metabolism on bioaccumulation potential. If the revised "B" estimate is greater than the regional criteria of interest, then a risk assessment may be performed. If the revised "B" value is less than the criteria of interest, consideration should be given to extrapolating the metabolic data across species (e.g., using PBPK modeling) and using these data as part of a "Weight of Evidence" approach to explain why the compound of interest is not bioaccumulative. If necessary, additional studies may be performed to examine the potential effects of major metabolites.

Tier 5- Risk Assessment

If a compound still meets criteria for "B" it will then be important to move on to a more formal risk assessment. The overall objectives will follow a risk-based approach to understand the true potential for "B" in the environment, and conducting an *in vivo* test (e.g., OECD 305) or field studies may be required. In this effort, the database of species "B" values may need to be expanded to include several levels in the food chain. It will be important to combine exposure, potential for absorption and metabolic clearance with relevant organism or population level data to evaluate the risk to organisms in the environment, especially predatory animals, based on the "B" evaluation.

Figure 1 - Overall "B" Decision Tree

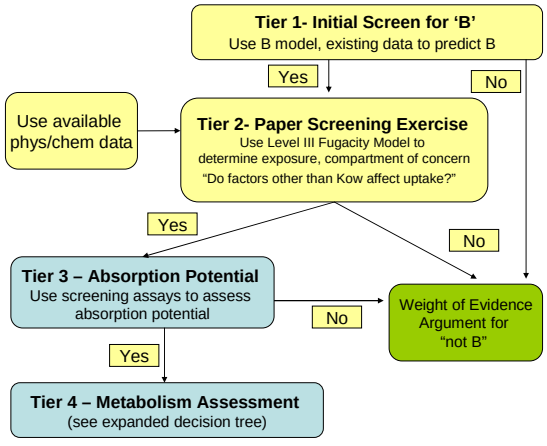
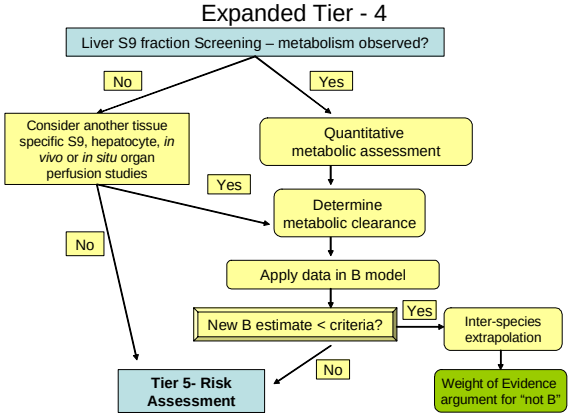


Figure 2- Metabolism Assessment Decision Tree
Expanded Tier - 4



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Development of a Strategy to Assess the Potential of *In Vitro* Methods to Predict Bioaccumulation

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Session Charge for HESI SETAC In vitro ADME Workshop (4 March 2006)

Design a research strategy that will lead to the determination of how and when diverse *in vitro* methods may be used to predict bioaccumulation in fish. Results of this session were needed to provide background and status information for the upcoming HESI sponsored workshop in Den Haag, The Netherlands (conducted prior to SETAC-Europe, May, 2006) and as an outline for a future publication.

Summary

There was a consensus that while in vitro methods provide great potential to estimate physical/chemical properties important for 'B' estimations, they will require further evaluation to verify their ultimate usefulness. The group believed this was best done via demonstration projects. Demonstration projects involve testing selected chemicals in abiotic and biotic systems that produce partitioning, absorption, and metabolism data; these data are scaled up and put into a generic fish bioaccumulation model (Arnot and Gobas, 2003) to estimate BCF/BAF ('B'). The 'B' model predictions incorporating the abiotic and biotic system data are ultimately compared to measured 'B' data. The following provide a brief description of the key tasks and methods that will be employed in conducting the demonstrations.

Selection of Chemicals

The potential success of any *in vitro* method can only be assessed in the context of the method choices available and type of chemical properties. Solubility, sorption, volatility, potential for biotransformation, metabolic pathways, availability of analytical methods and existence of reliable 'B' data (with species noted) are all factors that can affect the choice of chemicals to be evaluated. Table 1 provides a 'read-across' of the diverse factors that may be used to select chemicals to demonstrate how in vitro methods might be used in B assessments.

Table 1. Factors that can be used to select chemicals for a demonstration project.

Chemical	'B' (Kow-only)*	Measured 'B'**	Predicted *Kmet***	Species	Predicted Metabolic Pathway(s)****	Analytical Method Available*****
W	High	< Kow-only	High	From measured 'B' data	Phase I and II	
X	High	~ Kow-only	Low		Phase I	
Y	Low	< Kow-only	High		Phase I and II	
Z	Low	~ Kow-only	Low		None	

* 'B' (Kow-only) = predicted 'B' from a computer model using Kow as the only input and assumes no metabolism.
** 'B' is a measured value from reliable tests, e.g., OECD 305E BCF test, or field monitoring study.
*** 'Kmet', rate of whole-fish metabolism that is either quantitatively measured in vivo or in vitro (with scale up), or qualitatively estimated by subtracting measured 'B' from predicted 'B'.
****Metabolic pathways for mammals are available in text books and can be estimated by computer models, e.g.,TIMES, METEOR. However for fish, metabolism models are not available, hence potential pathways could be proposed based on best professional judgment (M James, K Kleinow, J Nichols and I Schultz volunteered to provide more details on this selection factor for interested parties).
***** A chemical's priority for selection is reduced if significant analytical method development is needed.

Systems that Describe Exposure and Dose

Suspected 'B' chemicals are typically highly sorptive, hence a primary route of exposure for fish to 'B' materials is via ingestion in the real world. Systems that can provide measures of sorption or bioavailability are critical for the proper estimation of 'B' and for understanding the results of various in vitro methods. These systems can be Abiotic or Biotic. An abiotic system typically refers to a solid phase that test chemicals absorb or adsorb to, and can be used to measure sorbed and free (soluble, unabsorbed) fractions. Biotic systems provide measures of transference across biological barriers (e.g., lumen, blood brain barrier). While several abiotic systems were discussed, two were favored by the session participants: EVA (ethylene vinyl acetate film) and SPME (solid phase micro-extraction). Since free and sorbed concentrations in the diverse in vitro methods are necessary for proper interpretation, EVA and SPME should be used in: sub-cellular and cellular media, water (for fish tests) and fish blood. Caco-2 and fish intestinal preps were the two biotic systems discussed. It was noted that although expert techniques are required to extract the tissue and conduct the test, only the intestinal preps are derived from fish and therefore thought to produce permeability data directly related to whole fish.

Subcellular Systems

Three different subcellular systems were discussed for metabolism studies: liver S9, microsomes, and homogenates. Only S9 and microsomes were considered worthy of further consideration because of the assays' higher degree of sensitivity and thus, measurability, of biotransformation. These systems are viewed as screening tools to assess the potential for biotransformation in higher biological systems (cellular, tissue and whole fish). While biotransformation (as metabolite generation and/or loss of parent material) rates can be measured in these test tube fractions, the consensus was that results should best be communicated as positive/negative (yes/no) or binned (high/medium/low) potentials. It was recommended that the same species of fish be used to compare utility of an in vitro test with in vivo measurements. For example, if a reliable measured 'B' value from Rainbow trout is used, then microsomal and S9 fractions from Rainbow trout should be used to evaluate how metabolism affects 'B' potential. Alignment on protocols (how to create the subcellular fractions, incubation temperatures, etc) will be necessary, particularly as investigations per fish species are compared across the diverse chemicals tested in

different laboratories. Efforts should be made to measure free and sorbed fractions in media. Parent chemical loss rates should be based on total and free fractions.

Cellular

Since 'B' materials are likely to enter fish via the intestine, there is a long-term need to assess the importance of intestinal uptake and metabolism on 'B' in fish. However at this time, fish intestinal preps are less commonly used, requiring method development. On the other hand, the use of fish hepatocytes to assess biotransformation is growing rapidly. Primary hepatocytes have increased realism in estimating 'B' beyond subcellular liver based preps because they include membrane transport (active and passive mechanisms). Their use has been primarily limited to only a few labs and species (Common carp and Rainbow trout). A current limitation of using hepatocytes is the need to have fresh fish cultures and their small tissue yield. To facilitate method transfer, there is a need to investigate the development of cryopreserved fish hepatocytes. Presently, cryopreserved hepatocytes from lab mammals and humans are commercially available. Parent chemical loss rates (hepatic clearance) should be based on total and free fractions.

In Situ Isolated Liver Preparations

Isolated liver preps provide the greatest integrative measure of uptake, distribution and biotransformation of chemicals in the liver. These preps have only been developed for catfish and Rainbow trout, species that have encapsulated livers and clearly defined hepatic and portal blood vessels. To enable a 'read-across' of methods exposure should be based on free and total fractions as dosed via blood.

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