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HESI Bioaccumulation Project Committee Workshop Summary

"Moving Bioaccumulation Assessments to the Next Level: Progress Made and Challenges Ahead"

February 8 – 10, 2011
Alexandria, VA

EXECUTIVE SUMMARY

An international workshop was held February 8-10, 2011 in Alexandria, Virginia, to discuss the state-of-the science and perspectives in bioaccumulation ('B') assessment. The purpose of this workshop was to summarize recent findings, prioritize research needs, and identify regulatory issues not being addressed by ongoing or planned work. The workshop was attended by scientists from academic, government, and private sector institutions, as well as risk assessors representing Environment Canada, the European Union, and the U.S. Environmental Protection Agency. An agenda from the workshop and list of participants are available online at:
http://www.hesiglobal.org/i4a/pages/index.cfm?pageid=3568

This report summarizes the results of the workshop and identifies priority needs in 'B' assessment. The document begins with a summary of five Overarching Themes identified during final wrap-up discussions. These themes, which cut across all major topic areas, are:

- Improved, open data access
- Development of benchmarks for methods and processes
- Determination of the domain of application for each method
- Addressing uncertainty in 'B' assessments
- Increased dialog with the regulatory community as a means of promoting acceptance of new data and methods

The remainder of the report is divided into sections that detail discussions and conclusions within four topic areas: 1**)** Predictive Models and Data Access; 2**)** *In Vitro* Methods; 3**)** *In Vivo* Methods; and 4**)** Predicting Bioaccumulation in the Field. The following represents a summary of high priority needs identified in each of the four areas. Additional detail is provided in the body of the report.

Predictive Models and Data Access

- Improve and expand on existing databases relevant to "B' assessment
- Enhance linkages between various 'B' models, as well as *in vitro* and *in vivo* data
- Enrich understanding of ADME processes in selected species representing a range of trophic levels
- Expand available 'B' models to include both aquatic and terrestrial organisms

In Vitro Methods

- Conduct additional research to address issues of variability and domain of applicability with developed methods (e.g., S9 metabolism assay, primary hepatocytes)
- Improve physiological data base for and validate existing *in vitro-in vivo* extrapolation models
- Develop novel methods using additional tissues or cell lines (e.g., gill and gut) to better predict chemical uptake and metabolism
- Utilize benchmarking compounds in evaluating novel approaches

In Vivo Methods

- Continue research on dietary feeding protocols and "minimized" aqueous exposure test designs
- Optimize the utility of each assay based on scientific, statistical, and economic questions
- Further evaluate the utility of chemical benchmarking approaches

Predicting Bioaccumulation in the Field

- Increase focus in the area of terrestrial bioaccumulation
- Execute well-designed comparative studies to improve extrapolation from the laboratory to the field
- Address statistical, design, and measurement challenges associated with the use of TMFs to improve their utility in the regulatory community and as a tool for designing field experiments.

The HESI Bioaccumulation Project Committee is working to prioritize key questions discussed at the workshop and develop a research plan for the next three years. A HESI steering team will be formed in the next few months comprised of members with expertise in the four research areas discussed above in addition to key regulatory representatives. This steering team will lead a concerted effort to increase connectivity amongst the research areas, initiate communication with the regulatory community, and ultimately, develop a weight of evidence scheme for 'B' assessment.

If you are interested in being involved with the HESI Bioaccumulation Project Committee work, please contact Dr. Michelle Embry (membry@ilsi.org). Additional information on the Bioaccumulation project can be found on the HESI website: www.hesiglobal.org

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I. Overarching Themes – Weight of Evidence for 'B'

Workshop participants were divided into four groups that followed the general tiered 'B' structure categories for the first two discussion sessions. They then convened for a final session which addressed how the various approaches used to assess 'B' can be integrated to develop an overall weight of evidence (WoE) approach. Current 'B' assessment strategies (Figure 1, REACH guidance, de Wolf et al., 2007) are structured in a tiered, linear fashion moving from simple modeling to field monitoring. Although it is recognized that the various methodologies are inter-connected, this linear approach does not adequately capture the importance of data integration and method development in each of the four fields providing 'B' data (Figure 2).

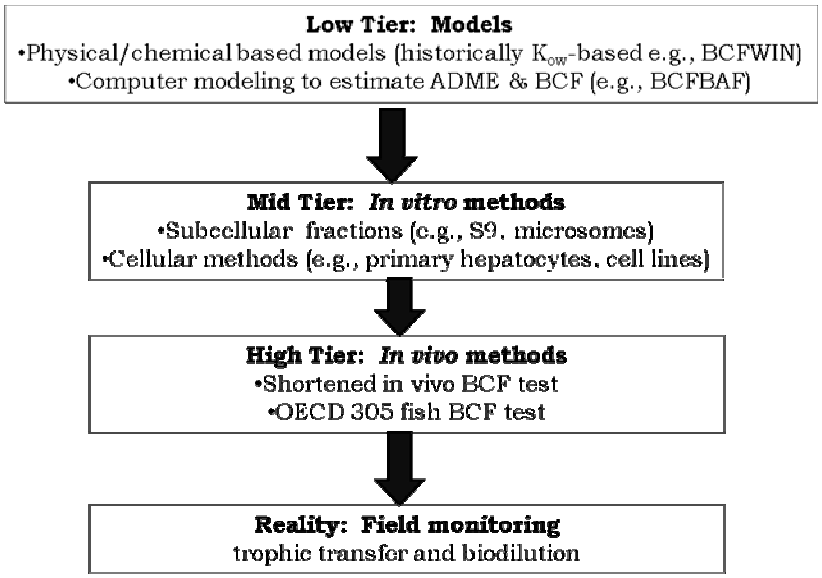

Figure 1: A typical tiered approach to 'B' assessment

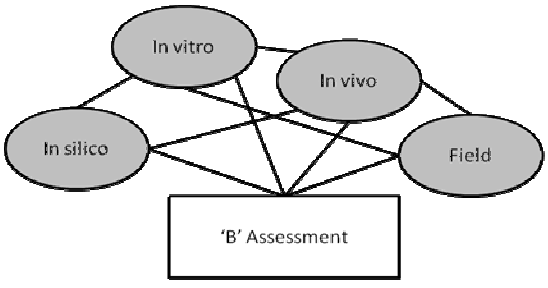

Figure 2: An integrated 'B' assessment approach, recognizing the connectivity between the various types of data as well as the ability for method development advances in one area to impact others.

Five themes emerged that highlight the need for better connectivity and integration between the various methodologies and tiers, all of which comprise components of a weight of evidence approach for B assessment.

- Increased access to data: Facilitation of increased data sharing and/or database development to bridge and integrate the various 'B' assessments- *in silico* – *in vitro* – *in vivo* – field

- Development of benchmarks: As new methods are developed, it is important to provide a standardized basis, such as benchmark chemicals, for evaluating these procedures. Selection and use of these chemicals may differ for each procedure. For instance, certain PCBs can be highly appropriate for benchmarking bioconcentration/bioaccumulation measures, yet are inappropriate for *in vitro* studies due to testing difficulties (low solubility, adsorption to glassware, etc.) and lack of metabolism. This topic arose primarily within the context of *in vitro, in vivo*, and field discussions.

- Domain of applicability: For *in silico, in vitro,* and *in vivo* methods, domains of applicability must be determined to provide guidance on use of the method for specific classes of chemicals.

- Addressing uncertainty: Increasing certainty in 'B' assessments is dependent on the level of confidence achieved with the various methodologies available. This confidence may be improved via standardization of specific assays, validation studies, and generation of data for chemicals possessing a wide range of attributes. Quantitative approaches such as Bayesian statistics might be used to address uncertainty and were highlighted as a key need. The specifics on how this might be realized for each set of data is an area for future discussion.

- Continued dialog and acceptance by regulatory agencies: Active communication with the regulatory community about advances and development of new methodologies is a critical need, and can be coupled with the themes described above. This communication should highlight how and why any new method can be used to improve 'B' assessment and effectively demonstrate linkages between the various tiers (e.g., *in vitro* to *in vivo*, etc.). The success of a tiered 'B' assessment will largely depend on the willingness of regulators to accept non-*in vivo* data.

Summary Recommendations

The five themes listed above represent critical features of a research program designed to support a weight of evidence approach for 'B' assessment of chemicals. An effort should be made to integrate these themes as a component of work performed in each of the research areas. In addition, an ILSI/HESI steering team for the project will be formed, comprised of members with expertise in the four research areas plus key regulatory representatives. This steering team will lead a concerted effort to increase connectivity amongst the research areas, initiate communication with the regulatory community, and ultimately, develop a WoE scheme for 'B' assessment.

II. Predictive Models and Data Access

Need for Improved Models

Chemical mass-balance models for fish have long served as valuable tools in 'B' assessments. Most such models represent the organism as a single compartment, and rate constants that control uptake and elimination are understood in terms of relevant physiological, biochemical, and physical-chemical processes. For many compounds, accumulation results from simple membrane diffusion (gill and/or gut) and partitioning into tissue lipid stores. In such cases, empirically-based algorithms that predict these processes from chemical log K_{OW} may accurately predict measured accumulation metrics (e.g., BCF, BAF). In other cases, however, the "baseline" (log K_{OW}–based) model does not

predict observed accumulation. Processes that can result in deviation from this simple partitioning behavior include chemical metabolism, specific binding, and restriction (e.g., due to ionization) or facilitation (e.g., due to transporters) of membrane transport. Unfortunately, these processes are difficult to predict from simple chemical properties.

For this reason, there is considerable interest in development of *in vitro* systems that can be used to provide critical model inputs. The one-compartment model structure also may be insufficient to predict observed accumulation, particularly for chemicals that undergo substantial extrahepatic metabolism. Research is needed to develop *in vitro* methods required to support a new generation of *in silico* "virtual fish" models that improve upon existing approaches. These models must be evaluated for chemicals exhibiting a diverse range of properties to determine their domain of application and provide confidence in the validity of the predictions.

Recent Research

The two recent advances in model development which were presented at the workshop were the development and validation of a QSAR model for estimating the whole-body metabolism rate constant from chemical structure ("k_M-QSAR") and an *in vitro*-to-*in vivo* extrapolation model that predicts the BCF from *in vitro* S9 and hepatocyte metabolism data. The k_M-QSAR model is included in the most recent version of the U.S. EPA's EPI Suite™ BCFBAF software package (Ver.4.0 and 4.1). The BCFBAF software also has been revised to include a new regression model based on reviewed BCF data, and mass balance models for predicting BCFs and BAFs in three trophic levels of fish. The three trophic level BCF and BAF predictions can include the k_M-QSAR model predictions. The *in vitro*-to-*in vivo* extrapolation model is integrated into the same BCF mass balance modeling approach that is included in BCFBAF. Both of these efforts represent advances over the previous state of the science which relied primarily on predictions of bioconcentration from log K_{OW} only.

Summary Recommendations

Workshop participants recognized that the goal of the modeling work is to provide models that can be used with confidence to de-prioritize chemicals for 'B' assessment. The goal is not necessarily to predict 'B' with a high level of accuracy but rather to determine that 'B' is unlikely to be a concern in a regulatory context. Thus, the discussion focused on what is needed to improve this type of assessment, how to evaluate one's relative confidence in the result, and what research is needed to accomplish these goals.

The availability of a reliable *in vitro*-to-*in vivo* extrapolation model was identified as critical need for acceptance of *in vitro* methods in 'B' assessments. Work that still is needed includes expanded validation of the model for a wider range of chemical classes, identification of the level of conservatism and confidence in the model, and improved parameterization.

The continued improvement of models for predicting bioconcentration and bioaccumulation depends on progress in several research areas. A critical need is to improve and expand existing databases used for 'B' assessment including data on relevant chemical, biological, and biochemical processes. These databases should focus on critically reviewed studies and might include information pertaining to chemical uptake from the gastro-intestinal tract (i.e., dietary assimilation efficiency), tissue metabolism, and chemical uptake from water. Thus, the recommendation was that additional data be sought and that work is funded to facilitate this effort.

Building on these improved and expanded databases, several modeling and data analysis tasks were identified which include:

- Improve the current BCFBAF model and expand the tested chemical space by further exploitation of existing data
- Improve metabolism prediction models by developing and evaluating new k_M-QSAR models and comparing results using different QSAR development techniques
- Explore relationships between hepatic and extra-hepatic metabolism
- Improve gill uptake and elimination predictions to reduce uncertainty in metabolism rates estimated from *in vivo* laboratory data (measured BCFs and/or observed rates of depuration)
- Improve chemical assimilation efficiency models, i.e., from diet and water
- Improve partitioning models, especially for chemicals where K_{OW} is not a good predictor of partitioning

A second major area of research is to improve the linkages between models (e.g., BCF mass balance, k_M-QSAR, *in vitro*-to-*in vivo* extrapolation), with the ultimate goal of developing a fully integrated virtual fish model. An exploration of these linkages could be used to identify and prioritize future research areas. The virtual fish model also could be used to improve (or as a component of) existing predictive models such as BCFBAF.

A third area of research is extrapolating the ability to metabolize chemicals in various organisms across species and trophic levels. There are fairly extensive metabolism databases available for mammals. The research question is whether these data can be used to develop a screen for whether a chemical is likely to be metabolized in aquatic organisms of concern. This capability is especially important for predicting metabolism in lower food web organisms and could be used to determine the likelihood of biomagnification within a food web. This information also can be used to develop pathway models that predict likely metabolic products from chemical structure.

The final area of research identified was expanding all of the models to include terrestrial and air-breathing organisms. This could be included as a component of previously described research but was recognized as a significant expansion of the original scope of the HESI Bioaccumulation Project Committee. Indeed, this expansion might warrant the formation of a working group focused specifically on this topic. Research tasks identified as part of this effort include developing a screen for metabolism in previously untested mammalian and bird species, and encouraging field monitoring for chemicals that based on current understanding could be bioaccumulative in terrestrial ecosystems.

<u>Priority Research Needs</u>
It was recognized that model development will involve both mining of existing data as well as generation of new data. An increased effort should be made to coordinate model development needs with collection of new *in vitro* and *in vivo* data, creating linkages between modelers and data generators.

Highest Priority

- Expanded validation of the *in vitro*-to-*in vivo* extrapolation model including improved parameterization
- Expansion and improvement of bioconcentration and bioaccumulation databases, including obtaining more existing 'B' data, i.e., Japanese METI data, and other bioaccumulation parameters such as gill and gut assimilation efficiencies
- Improve dietary assimilation efficiency models because for many chemicals of concern this is the primary route of uptake

- Develop and validate k_M-QSAR models
- Improved ability to extrapolate metabolism information across species and trophic levels, including development of screening methods for identifying chemicals that are likely to be metabolized
- Develop a "consensus" terrestrial food web bioaccumulation model from existing terrestrial food web models

Lower Priority

- Improvement in BCFBAF model and its associated k_M model
- Improvement in gill uptake predictions
- Development of virtual fish models
- Development of metabolism pathway models

III. *In Vitro* Methods

Need for Completion and Delivery of *In Vitro* Methods for Bioaccumulation

In breakout group discussions, workshop participants reviewed recent progress toward development of *in vitro* test methods and discussed what it would take to gain acceptance of these methods by regulators. Due to the availability of commercial cryopreserved fish S9 fractions and the ease of conducting *in vitro* biotransformation testing with these fractions, an extensive pre-validation study using rainbow trout S9 fractions was coordinated by HESI. This effort led to the development of a standardized protocol and evaluation of nine test compounds in five different laboratories. However, continued evaluation of these "optimized" methods is necessary, in addition to increased focus on key issues such as observed high inter-laboratory variability and its impact on predicting 'B'.

While these S9 *in vitro* methods are proving to be highly useful as screening methods, it is likely that *in vitro* tests using primary hepatocytes will contribute an additional degree of realism and refinement to the biotransformation measurement. This is due to the fact that hepatocytes incorporate membrane-driven processes in addition to the active Phase I and II biotransformation enzymes found in S9 fractions. Unfortunately, the majority of hepatocyte studies conducted to date have been performed using fresh primary cultures that require adjacent fish holding facilities. Therefore, few laboratories have the capability to conduct such studies. In order to increase the number of laboratories that can conduct *in vitro* hepatocyte tests, research has been focused on the feasibility of cryopreserving fish primary hepatocytes. As with the S9 fractions, additional work towards the development of a robust cryopreservation method is needed. A round robin study of cryopreserved fish hepatoctyes using standardized methods is anticipated in the near future. Importantly – at this time, there are no commercial suppliers with continual availability of fish tissue systems.

A rainbow trout intestinal epithelial cell line (RtgutGC) is available (Kawano et al., 2010) and has been successfully adapted to grow on a permeable support (K. Schirmer, unpublished results). This first step is critical for development of a cell-line based intestinal barrier model for fish, similar to the human Caco-2 model. Fish gill cell lines are widely available, but to date have not been used for bioaccumulation assessment (Lee et al., 2009). Test chemical bioavailability needs to be considered when using *in vitro* cell-based assays. One promising approach is to dose cells using passive dosing systems. Examples of this approach and lessons learned can be re-applied from research on mammalian cell lines, since much of this work has already been done.

Summary Recommendations and Priority Research Needs

To advance the utilization of *in vitro* methods in predicting chemical accumulation by fish the following strategies were suggested:

- Funds are needed to continue laboratory work to:
  - Resolve/explain inter-laboratory variability in S9 study results, including the impact of this variability on overall bioaccumulation estimates
  - Complete the development of cryopreservation techniques for trout hepatocytes and conduct biotransformation round robin studies
  - Continue to initiate research in new tools such as gill and gut cell lines
  - Further develop *in vitro* to *in vivo* extrapolation models by collecting additional physiology and binding parameter data

- To gain regulatory acceptance and to propagate trust in the methods the following activities should be undertaken:
  - Demonstration of test relevance by demonstrating biological relevance and extrapolation of *in vitro* results to *in vivo* BCF test results when possible. This will involve close interaction with researchers developing new modelling tools (Section II) as well as those developing new *in vivo* methods (Section IV).
  - Demonstration of test reliability through examination of inter- and intra laboratory reproducibility
  - Identification of species differences and discussion of species selection
  - Identification of chemical applicability domains via data mining and development of a reference chemical list
  - Assessment of uncertainty in each of the new methodologies
  - Development of a benchmarking approach to compare methods and species
  - Development of SOPs for each method along with support for training and education as new methods become available

IV. *In Vivo* Methods

Need For Bioaccumulation Test Method Revision

Several published methods for measurement of chemical bioconcentration in fish have been provided (U.S. EPA 1996; OECD 1996; ASTM 2007). Although they differ in detail, each method prescribes a relatively intensive sampling schedule (typically 9 or more sampling times) and requires a large number of animals (typically > 100, assuming two tested concentrations). Given the high cost of performing such tests as well as growing concern for animal use in regulatory assessments of chemicals, there is strong motivation to develop refined or new methods which are less expensive, require fewer animals, and yield more data in a shorter period of time.

In breakout group discussions, participants reviewed recent work on development of new bioaccumulation test methods using live animals and identified research needed to support continued development of these methods. A description of recent work is provided as a means of providing context for a set of summary recommendations that derive from proposals made by various participants. Issues related to continuing improvement and regulatory adoption of new assessment methods are identified in separate sections, followed by a prioritized list of research needs.

Recent Research

Recent work toward development of new bioaccumulation test methods consists of two complementary efforts. The first is focused on development and evaluation of a dietary exposure method. This work builds on methods developed by ExxonMobil Biomedical Sciences (EMBSI) and was motivated by the challenge of conducting aqueous exposures with very hydrophobic compounds. A ring test designed to evaluate method performance in multiple laboratories is ongoing in the context of the OECD Test Guidelines program.

The second effort is focused on modifications to current aqueous exposures methods. A "minimized" test design that involves limited animal numbers was initially proposed by Springer et al. (2008). Similar proposals have appeared in draft revisions to the OECD 305 test guidelines (Merckel 2011). Other work supported by CEFIC LRI has been designed to "optimize" new test methods by using passive dosing methods and chemical benchmarking approaches (McLachlan 2011).

Summary Recommendations

Workshop participants endorsed the following measures which are designed to improve the quality and comparability of BCF measurements obtained using "minimized" or "full" study designs.

- Kinetic BCF values should be corrected for the dilution of chemical residue in fish tissues by growth of the fish. The available method for performing this growth correction involves calculating the BCF value using kinetic rate constants [i.e BCF = uptake rate constant / (depuration rate constant – growth rate constants)], although an alternative method has been suggested recently (Environment Agency, 2011). No accepted method is available for correcting the steady-state BCF for growth dilution.
- BCFs should be normalized to organism lipid content.
- Preliminary investigations should be conducted to estimate the optimum lengths of uptake and depuration phases for a BCF study. It is particularly important that the depuration period be longer than one depuration half-life.
- Exposure concentrations should be well below aqueous solubility and/or levels known to exert toxic effects. Ideally, test concentrations should be informed by "environmentally relevant" levels.
- For high log K_{OW} compounds, consideration should be given to estimating the unbound chemical fraction in water. This can be done using existing algorithms if POC/DOC levels in the water are known (Arnot and Gobas 2004). Alternatively, it may be possible to measure the unbound chemical fraction using passive sampling methods.

Outstanding Issues

Adoption and use of new bioaccumulation test methods is limited by several outstanding issues:

- *Dietary vs. aqueous dosing.* Guidance is required on when to use different dosing methods. This issue relates in part to ease of testing (especially for very hydrophobic compounds) as well as current regulatory reliance on BCF values. A need exists for methods to interrelate aqueous and dietary test results. Participants noted that future regulations may accept (and perhaps give preference to) biomagnification (BMF) values from feeding studies, but currently numerical drivers in chemical regulations use BCF.

- *"Minimized" BCF test design.* What design provides the optimal balance between resource use and quality of study results (defined here as comparability to the full BCF test)?
- *Benchmarking.* Limited work (McLachlan 2011) suggests that chemical benchmarking approaches may improve BCF test results.
  - What are the processes that we would like to benchmark and how should benchmarking compounds be selected?
  - A chemical benchmark in a bioconcentration experiment is a chemical with a "known" BCF that is dosed to the fish at the same time as the target chemical.
  - The ratio of the experimentally determined BCFs of the target chemical and the benchmark chemical is then multiplied by the "known" BCF of the benchmark chemical to yield a best estimate of the BCF of the target chemical. This approach can correct for many sources of variability in BCF experiments such as species differences, ventilation rates, and some kinds of sampling and analytical errors.

- *Benchmarking vs. other approaches.* Because it requires the analysis of two compounds, benchmarking imposes an additional cost on the typical BCF assay. This raises the question: are limited resources better spent on benchmarking or incorporation of additional samples?

- *Testing of chemical mixtures.* By testing chemical mixtures it may be possible to provide more data at lower cost, relative to chemical-by-chemical testing approaches. However, a substantial number of compounds induce or inhibit enzymes responsible for their own metabolism and/or metabolism of other substrates. Chemicals with shared metabolic pathways also may exhibit competitive inhibition. Given these considerations, is it advisable to test chemical mixtures if these compounds are known or suspected of undergoing metabolism?

- *Multi-exponential and/or non-linear kinetics.* The "one box" model commonly used for BCF modeling assumes first-order mono-exponential kinetics. Is this always a good assumption? If not, under what conditions does it fail and what modifications to current modeling approaches are needed?

<u>Future Use of New *In Vivo* Test Methods</u>

Assuming future adoption of new *in vivo* test methods, participants identified several issues related to their use in a regulatory environment.

- How will new methods be used within the context of proposed tiered and/or WoE approaches to bioaccumulation assessment?
- What criteria would be used to recommend a specific test design?
- Would equivocal results from a "minimized" BCF test trigger the need for a full test?
- What if depuration data evidence multi-exponential kinetics (see above)?
- What if aqueous and dietary tests appear to give contradictory results?
- When are BCF data from one or a few species sufficient/insufficient, especially for compounds that undergo substantial metabolism (and for which species differences might be expected)?
- How do you weight or otherwise integrate *in vitro* and *in vivo* test results in a tiered and/or WoE approach?

Priority Research Needs

Workshop participants identified and prioritized the following research needs.

Highest priority

- Continue research on both dietary feeding protocols and "minimized" aqueous exposure test designs. Although these assays may ultimately be applied to different types of compounds, work involving the same species and chemicals should be conducted to provide a basis for making comparisons among the two approaches.

- Rigorously address scientific, statistical, and economic questions pertaining to each assay as a means of "optimizing" their utility. This information is needed to evaluate trade-offs in terms of assay performance (e.g., precision and accuracy of BCF/BMF determinations), as well as cost and animal usage.

- Further evaluate the utility of chemical benchmarking approaches, noting the potential trade-off s identified above.

Lower priority

- Evaluate species differences in bioaccumulation, especially for compounds that undergo substantial metabolism. How large are these differences? Are these differences predicted by *in vitro* assay results?

- Evaluate the possibility of testing chemical mixtures, especially for compounds that undergo substantial metabolism. (McLachlan 2011).
  - Will several highly metabolized chemicals tested singly accumulate to a different degree if tested as a mixture?
  - Does the answer to the above question depend on temporal considerations (given possible induction/inhibition effects) and/or the concentrations of tested substances (given possible saturation of metabolic pathways)?

- Evaluate the possibility of multi-exponential kinetics.
  - What are the circumstances under which this could occur?
  - Is there evidence for this in the literature?
  - How could inappropriate application of a one-compartment model to data of this type influence predicted BCFs/BMFs?
  - Could we expect multi-exponential kinetics to yield different results for dietary and aqueous exposure protocols, especially for compounds that undergo substantial metabolism?

V. Predicting Bioaccumulation in the Field

Bioaccumulation Metrics

Well-known metrics of bioaccumulation include the BCF, BAF, BSAF (biota-sediment accumulation factor), BMF, and TMF (trophic magnification factor). Although closely related, these metrics describe different processes and outcomes, and scaling from one to another may be difficult. In general, regulatory authorities tend to rely on laboratory BCF and BAF values when evaluating a compound's potential to accumulate in aquatic biota. Not surprisingly, HESI research projects funded to date reflect this emphasis. It may be argued however, that the most relevant measures of chemical accumulation in a field setting are the BMF and TMF. An examination of TMFs developed from field data may

therefore provide an improved means for identifying bioaccumulative substances (Gobas et al., 2009). Still others have pointed out potential challenges associated with this approach. Thus, TMFs may be highly influenced by attributes of the ecosystem being sampled. Processes that can substantially impact measured TMF values include biodilution at lower trophic levels and differential metabolism throughout the food web.

In initial breakout discussions, participants reviewed recent work in the area of field sampling and lab-to-field extrapolation, and identified strengths and limitations of this work as well as information gaps and needed improvements. In a follow-up discussion, participants developed a prioritized list of research projects needed to support continued development of these methods.

Summary Recommendations

- *Terrestrial Food Webs in Regulatory Context.* The development of terrestrial food web models was viewed as a high priority. Although most bioaccumulation research has focused on the aquatic environment to-date, the terrestrial exposure route is important for both human and wildlife exposure and should be a major focus in the years ahead.

- *Lab-field Extrapolation.* The development and use of BSAFs (e.g., bioavailablity and the presence/absence of steady-state conditions) were highlighted because this metric has recently gained regulatory attention. Moreover, these BSAF-related issues can inform model development and can contribute to an increased understanding of the linkages between abiotic compartments and lower trophic levels. Models are effective tools for the design of field studies and may provide a means for understanding complex systems. However, understanding feeding ecology and temporal and spatial heterogeneity, such as algal blooms and seasonal variability, are key issues that need attention. Currently, good, well-designed comparative studies (lab-field-model) are lacking.

  Extrapolation from lab to field will be improved by:

  - Research on the distribution and type of Black Carbon (BC) in natural sediments and the effect of different sediment components on chemical absorption efficiencies from the abiotic compartment to lower trophic levels.
  - Characterizing food web structure, such as diet composition and abundance, as a means of better defining dietary uptake (Selck et al. 2011 IEAM online).

- *TMF in regulatory processes.* TMFs are widely used; however, there are numerous statistical, design and measurement challenges. The strength of this method is based on the fact that if a well-designed TMF study yields a TMF greater than 1, it provides convincing evidence of PBT (Persistence, Bioaccumulation, Toxicity) characteristics. On the other hand, TMF tends to be limited to legacy chemicals for which good analytical methods exist. A generic d15N enrichment baseline correction organism is needed as well as a standardized sampling strategy (among other parameters) such that the quality of the TMF can be confirmed before TMF can be recommended for regulatory use.

- *TMF as tool for designing field experiments.* The TMF is recommended as a tool for designing balanced field experiments (Borga et al., 2011; Conder et al., 2011 both: IEAM online).

- *TMF in the terrestrial food web.* The full *TM*F approach of assigning trophic levels via d15N of individual animals and for vegetation has not yet been applied to terrestrial biomagnification studies; however, Kelly and Gobas (2003) and Kelly *et al.*, (2007) have modeled it using basic trophic levels as has McLachlan (1996) for agricultural food webs.

Priority Research Needs

- Initiate new research on terrestrial bioaccumulation
    - Further evaluate the hypothesis that chemicals with log K_{OW} >2 - <5 and log $K_{OA} \geq 6$ can biomagnify in terrestrial systems
    - Continue with *in vitro* S9 metabolism research to obtain needed k_{met} values for use in models, with concerted effort to include terrestrial species.

- Improve benthic bioaccumulation models and increase understanding of transfer from abiotic to lower trophic levels to improve model parameterization. The suggested focus areas represent serious knowledge gaps in the literature.
    - Lab-to-field extrapolation:
        - Matched studies - comparison of bioassay (same organism and sediment as in field plus spiked sediment) versus field BSAFs.
        - Role of black carbon / labile organic matter, as well as matrix effects on bioaccumulation
        - Methods for determining black carbon
    - Benthic bioaccumulation models: Improve models by considering bioavailability from the sediment compartment
    - Potentially (lower priority): Passive samplers

- TMF-related work
    - A laboratory mesocosm "B metric study" is needed to estimate TMF values for newly developed chemicals.
    - Development of standardized approaches to field sampling would be helpful in obtaining field BMF and TMFs.
        - Criteria for balanced field design and a reference chemical approach could be established to help standardize field evaluation of chemical biomagnification and trophic level transfer.
        - Research in the area of d15N enrichment factors is needed to set the "spread" of trophic levels, as generic d15N enrichment factors are unlikely to apply equally across a variety of food webs.
        - Consideration should also be given to the utility of including evaluation of a reference chemical with well characterized TMF behavior (e.g., PCB153) to both help establish d15N enrichment factors and food web interactions.
- Lab-to-field extrapolation
    - Further investigate the role of feeding ecology as well as temporal and spatial heterogeneity as determinants of bioaccumulation (both aquatic and terrestrial)
    - Further investigate the role of dietary composition and prey abundance in bioaccumulation predictions

REFERENCES

American Society for Testing and Materials. 2003. E1022-94. Standard guide for conducting bioconcentration tests with fishes and saltwater bivalve mollusks. ASTM International, West Conshohoken, PA.

Arnot,JA, Gobas FAPC. 2004. A food web bioaccumulation model for organic chemicals in aquatic ecosystems. *Environ Toxicol Chem.* 23:2343-2355.

Borgå K., Kidd K., Berglund O., Conder J.M., Gobas F.A.P.C., Kucklick J., Malm O., Powell D.E., Muir D.C.G. 2011. Trophic Magnification Factors: Impact of Ecology, Ecosystem and Study Design. *Integ Environ Assess Manage* (in press).

Burkhard LP, Cowan-Ellsberry C, Embry MR., Hoke RA, Kidd KA. 2011. Introduction to special series. Bioaccumulation data from laboratory and field studies: Are they comparable? *Environ Toxicol Chem* (in press).

Conder JM, Gobas FAPC, Borgå K, Muir DCG, Powell DE. 2011. Characterizing Bioaccumulative Potential of Chemicals Using Trophic Magnification Factors and Related Measures. *Integ Environ Assess Manage* (in press).

De Wolf, W, Comber M, Douben P, Gimeno S, Holt M, Léonard M, Lillicrap A, Sijm D, van Egmond R, Weisbrod A, Whale G. 2007. Animal use replacement, reduction, and refinement: development of an integrated testing strategy for bioconcentration of chemicals in fish. *Integr Environ Assess Manag.* 3:3-17.

Environment Agency of England and Wales. 2011. In: Crookes M, Brooke D. *Estimation of fish bioconcentration factor (BCF) from depuration data* (in press), http://publications.environment-agency.gov.uk/

Gobas FAPC, de Wolf W, Burkhard LP, Verbruggen E, Plotzke K. 2009. Revisiting bioaccumulation criteria for POPs and PBT assessments. 2009. *Integ Environ Assess Manag.* 5:624-637.

Kawano A, Haiduk C, Schirmer K, Hanner R, Lee L, DixonB, Bols N. 2010. Development of a rainbow trout intestinal epithelial cell line and its response to lipopolysaccharide. *Aquacult Nutr* doi: 10.1111/j.1365-2095.2010.00757.x

Kelly BC, Ikonomou MG, Blair JD, Morin AE, Gobas FAPC. 2007. Food Web Specific Biomagnification of Persistent Organic Pollutants. *Science.* 317: 236-239.

Kelly BC, Gobas FAPC. 2003. An Arctic Terrestrial Food-chain Bioaccumulation Model for Persistent Organic Pollutants. *Environ Sci Technol.* 33: 2155-2511.

Lee LEJ, Dayeh VR, Schirmer K, Bols NC. 2009. Applications and potential uses of fish gill cell lines: examples with RTgill-W1. *In Vitro Cell Dev Biol—Animal.* 45:127–134.

McLachlan M. 1996. Bioaccumulation of Hydrophobic Chemicals in Agricultural Food Chains. *Environ Sci Technol* .30: 252-259.

McLachlan M. 2011. Benchmarking and passive dosing/sampling in fish BCF experiments. Plenary presentation to Workshop participants.

Merckel D. 2011. Update on OECD 305 TG revisions. Plenary presentation to Workshop participants.

Nichols J., Erhardt S., Dyer S., James M., Moore M., Plotzke K., Segner H., Schultz I., Thomas K., Vasiluk L., WeisbrodA. 2006. Use of in vitro absorption, distribution, metabolism, and excretion (ADME) data in bioaccumulation assessments for fish. *Human Ecol. Risk Assess.* 13:1164-1191.

Organization for Economic Co-Ordination and Development 1996. Bioaccumulation: Flow-through Fish Test. OECD guideline for the testing of chemicals No. 305. OECD, Paris, France.

Selck H., Drouillard K., Eisenreich K., Koelmans A.A., Palmqvist A., Ruus A., Salvito D., Schultz I., Stewart R., Weisbrod A.V., van den brink N.W., van den Heuvel-Greve M. Explaining variability of bioaccumulation measurements between laboratory and field using a modelling approach. *Integrated Environmental Assessment and Management.* Accepted.

Springer, TA, Guiney, PD, Krueger, HO, Jaber, MJ. 2008. Assessment of an approach to estimating aquatic bioconcentration factors using reduced sampling. *Environ. Toxicol. Chem.* 27:1171-1180.

U.S. Environmental Protection Agency 1996. Ecological Effects Test Guidelines. OPPTS 850.1730 Fish BCF. Public Draft. Office of Prevention, Pesticides and Toxic Substances, Washington, DC.