

**Summary of Cefic-LRI Sponsored Workshop:
Recent Scientific Developments in Bioaccumulation Research**

Workshop Report

**Prepared for
Cefic-LRI**

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Bioaccumulation Assessment: Key Points

- Current bioaccumulation regulations in most jurisdictions include only the bioconcentration factor (BCF) and the octanol-water partition coefficient (K_{ow}) for screening assessments. Methods for evaluating bioaccumulation continue to evolve and various other metrics have been proposed including the biomagnification factor (BMF), bioaccumulation factor (BAF), trophic magnification factor (TMF), and elimination half-life ($t_{1/2}$)^{1,2}.
- Environmental measurements are most relevant regarding a chemical's actual behavior in the environment; however, due to natural variability, field measurements, and hence field bioaccumulation data, can be quite variable. Environmental sampling that does not consider variability can translate to uncertainty, which reduces the precision of field-derived bioaccumulation metrics which may be important in a regulatory context. Models and guidance are recommended to develop consistent methods for collecting and interpreting field data.
- The BCF does not account for chemical uptake from the diet; therefore, it may not reflect the extent of bioaccumulation that would result from an environmental exposure. However, models can be used to relate the BCF to environmentally relevant metrics such as the BMF, BAF, and TMF in a straightforward manner³.
- Current science indicates that the biotransformation rate constant (k_B) represents the principal source of uncertainty in the bioaccumulation assessment of most chemicals with high bioaccumulation potential and k_B is a common element to all bioaccumulation assessment metrics. It is clear that more research to improve the quantification and estimation of biotransformation rates and pathways is critical for improving bioaccumulation screening assessments. *In vivo* k_B databases and *in silico* models (quantitative structure-activity relationships, QSARs) for predicting k_B from chemical structure have recently been established, but more k_B data are needed and a wider range of measurement techniques should be developed. *In vitro* and *in vitro*-to-*in vivo* extrapolation (IVIVE) methods and data to estimate clearance and k_B continue to evolve and offer a promising path forward. Since k_B is often the key determinant of existing and proposed bioaccumulation assessment metrics, it was suggested that k_B could be used as a new screening-level criterion for bioaccumulation assessment. A combination of estimates for k_B derived from different methods can be applied in a weight of evidence approach (WoE) to address uncertainty.
- A weight of evidence (WoE) is suggested for screening assessments (to the greatest extent possible, often with limited data) and certainly for definitive decision-making (cases in which more data are available or can be obtained). A WoE framework has been developed for biomagnification assessment¹. A complimentary framework and formal guidance for overall bioaccumulation assessment is suggested.

Introduction. The European Chemicals Agency (ECHA) hosted a workshop in Helsinki, Finland September 24, 2014 organized and sponsored by Cefic-LRI to communicate and discuss recent scientific advancements for assessing chemical bioaccumulation, including alternatives to animal testing. The general objectives of the workshop were to:

- (1) present and discuss recent developments in bioaccumulation science, including, but not limited to, ongoing and finalized Cefic-LRI projects, and
- (2) evaluate current bioaccumulation assessment practices for organic chemicals to identify optimal approaches based on the 'State of the Science'.

The workshop included 10 speakers and moderators and 75 additional participants representing academia, industry and governments. There was an introductory session, five technical sessions and a final summary session on bioaccumulation assessment. The workshop included active scientific discussions among speakers and participants. This document provides a summary of key topics raised and discussed in the workshop.

Technical Background. The first technical session of the workshop provided participants with an overview of scientific concepts, terminology and metrics used to describe bioaccumulation and existing regulatory approaches to control bioaccumulative chemicals. The session was chaired by Dr. Matthew MacLeod and featured a concise summary of the state of bioaccumulation science prepared and presented by Dr. Jon Arnot and Professor Frank Gobas.

The discussion began with a historical summary of the discovery of bioaccumulation of organic chemicals as an environmental problem and a potential problem for human health due to exposure through the food chain. The first bioaccumulative chemicals to be well characterized were persistent, hydrophobic and non-dissociating. Scientific study and regulatory approaches were initially focused on the bioconcentration of these substances from water to fish in laboratory tests. The bioconcentration of these chemicals can often be adequately predicted assuming equilibrium partitioning and using the octanol-water partition coefficient (K_{ow}) as a surrogate for partitioning between fish lipid and water. A more refined description of bioaccumulation of chemicals by fish evolved that described the process as a competition between rates of chemical uptake (from the environment and dietary exposure pathways) and elimination. Bioaccumulation science has now evolved toward developing *in vitro* and *in vivo* laboratory studies, *in silico* models and quantitative structure-activity relationships (QSARs) to quantify these individual competing processes, and mass balance and food web models to compare predictions with field observations.

The process-based approach to describing bioaccumulation has produced a toolbox of concepts and metrics that can be used to describe specific aspects of the bioaccumulation phenomenon. We can distinguish between bioconcentration

(chemical exposure from the ambient environment), biomagnification (chemical exposure from ingestion) and bioaccumulation (combined environmental and ingestion exposures). Related metrics include the bioconcentration factor, BCF (steady state ratio of chemical concentration in an organism and its ambient environment in absence of dietary exposure), biomagnification factor, BMF (steady state ratio of chemical concentration between an organism and its food), bioaccumulation factor, BAF (steady state ratio of chemical concentration in an organism and its ambient environment including dietary exposure), and trophic magnification factor, TMF (the average biomagnification factor in a food web that includes multiple trophic levels) (see detailed definitions elsewhere ¹). The elimination half-life ($t_{1/2}$) has also been proposed as a metric for bioaccumulation assessment ².

The process-based methods have shown that chemical bioavailability in the environment, the absorption efficiency of ingested chemicals, and the biotransformation rate are key factors determining the extent of bioaccumulation for most hydrophobic chemicals. The mass balance modelling framework makes it possible to integrate physical-chemical properties, observations from *in vitro* and *in vivo* laboratory studies, and field measurements in a cohesive manner ³. Many seemingly disparate types of bioaccumulation information can be assembled into a weight of evidence (WoE) approach that has several advantages over bioaccumulation assessments based on one type of information or results of a single test considered in isolation.

Field Data. The purpose of the session on field bioaccumulation data was to discuss the prospect of including bioaccumulation information from field studies in a WoE approach to bioaccumulation assessment. It is desirable to use field (monitoring) data to the greatest extent possible for bioaccumulation assessment because these measurements include environmentally relevant processes and the exposure and potential risk of chemicals occurs in the “real-world” environment. The application of field derived BMFs, BAFs, and TMFs has been suggested in a WoE approach for bioaccumulation assessment ¹.

Dr. Borgå and Dr. Leonards presented field research on the bioaccumulation of a range of legacy and emerging pollutants. They highlighted three recent papers of regulatory relevance for using field data for bioaccumulation assessment ⁴⁻⁶. One of their key observations is that much of the variability associated with field bioaccumulation data can be assigned to a number of factors including differences among ecosystems, ecology and physiology of the species from which the data are obtained. The selection of representative samples, the number of species included in a food web analysis and the number of samples collected per species are important factors that can reduce uncertainty and control the variance. By considering these issues when designing a bioaccumulation field study, some of this variability can be accounted for and thus excluded from the uncertainty of the data. A method to address uncertainty in trophic level estimation was presented ⁷. Steady state is an underlying assumption for field bioaccumulation metrics; however, steady state

may not exist in natural situations with variable conditions and migrating animals. Steady state is more likely achieved within a representative food web, and spatial and temporal analysis and uncertainty analysis are suggested for better characterizing and understanding the role of variability on field bioaccumulation. Initial temporal field studies and spatial modeling studies suggest significant influence on the TMF; however, only minor effects were found and these did not affect the bioaccumulation assessment outcome ^{8, 9}. The application of a bioaccumulation model that considers spatial concentration differences in the environment was presented to demonstrate the effect spatial heterogeneity and species movement may have on the TMFs of PCBs ⁹. Dr. Gobas also presented results from a similar spatial food web bioaccumulation model to assess the effects of spatial concentration differences, species migration and biotransformation rates on the TMF. The modeling studies show that spatial factors and biotransformation can have a substantial influence on field derived bioaccumulation metrics. The session also noted the importance of further research on bioaccumulation in terrestrial systems and on the bioaccumulation of ionogenic chemicals such as acids and bases.

During discussions the workshop participants recognized that field bioaccumulation data make an important contribution to a WoE approach for chemical assessments. Natural variability and the inability to control environmental and biological processes that influence bioaccumulation provide challenges to the collection, interpretation, and regulatory use of field bioaccumulation data. Further research is needed to better understand the factors that contribute to the variability in field bioaccumulation metrics. The development of field study guidelines that can address uncertainty of field derived bioaccumulation metrics was recommended. Furthermore, the development of techniques (such as the fugacity ratio approach ¹) that allows the evaluation of bioaccumulation data from field, laboratory and modeling studies provides a strong basis for a WoE approach to bioaccumulation assessment.

Laboratory Data. Dr. Arnot began by describing efforts to collect and evaluate measured laboratory BCFs for fish. In their landmark paper, Arnot and Gobas ¹⁰ presented fish BCF data for 770 chemicals. Using defined criteria to identify recognized sources of uncertainty in BCF tests ¹¹ the authors identified a subset of BCFs that were of “acceptable” quality (2527 BCFs for 646 chemicals). A similar review of BAF data revealed 912 “acceptable” BAFs for 92 chemicals. Using the Canadian Domestic Substances list of 11,300 organic chemicals as a representative sample of chemicals in commerce the review suggests that acceptable quality BCFs and BAFs exist for roughly 3% and 0.2%, respectively. By focusing on high quality BCFs, the range of reported values for a given chemical may be substantially reduced. Moreover, many sources of uncertainty result in measured BCFs that underestimate true values. Dr. Arnot concluded by noting that BCFs from minimized test designs ^{12, 13} are well-correlated with BCFs using “full” testing protocols. This supports the use of a minimized test design (fewer animals, less cost) if specific criteria are met as outlined in recent BCF test guidance ¹⁴.

Dr. Arnot then described a recent effort to collect and evaluate laboratory BMF data for fish (Arnot and Quinn, in review). Data quality criteria were again derived from standardized test guidance ¹⁴ and the data were evaluated to assess their general reliability. A total of 313 BMFs were determined to be “reliable” or “reliable with restrictions” (acceptable quality). Among these were a number of BMFs that exceed 1, which is a commonly cited threshold of concern. Most BMF > 1 have been determined for contaminants that are known to be poorly biotransformed. Many studies designed to measure the BMF using kinetic methods depend critically on accurate determination of an elimination half-life ($t_{1/2}$). If the $t_{1/2}$ is determined from chemical concentrations it is important to account for growth effects for persistent hydrophobic chemicals, since growth “dilutes” the amount of chemical in the organism during depuration.

Several authors have compared different laboratory metrics of bioaccumulation obtained for the same chemical. For example, Inoue et al. ¹⁵ showed that while BCFs correlate positively with BMFs, BCFs exceeding 5000 (a commonly employed regulatory threshold) are not necessarily associated with BMFs greater than 1. Thus, a compound may or may not be classified as bioaccumulative depending on the data available, the bioaccumulation metric, and the chosen bioaccumulation criterion. In a follow-up study, Hashizume et al. ¹⁶ evaluated a number of different methods for predicting the rate constant for chemical uptake from water (k_1). Using the three “best” methods, the authors then used depuration rate constants from a set of BMF studies ($k_{T,diet}$) to predict BCFs for 12 study compounds (as the ratio $k_1/k_{T,diet}$). The predicted BCFs correlated reasonably well with measured values, although in several cases the bioaccumulation classification associated with a predicted value was different from that associated with the measured value. Growth correction of the $k_{T,diet}$ value was shown to improve the correlation of measured and predicted BCFs, again illustrating the importance of accounting for growth effects.

Dr. MacLeod began his presentation by using a model to address the question “when is *in vivo* data most needed for bioaccumulation assessment?” Within a modeling domain described by K_{OW} and the biotransformation rate constant (k_B), it was shown that for different regions different processes tend to dominate the outcome, defined in terms of the BMF. Thus, for low (< 4) log K_{OW} chemicals biotransformed at all but very high rates, gill elimination dominates and growth dilution is unimportant. For very high (> 8) log K_{OW} compounds, growth dilution and biotransformation both have the potential to dominate. However, BMFs for these compounds can be low due to lower dietary absorption efficiency. Perhaps the most interesting region is that defined by compounds with log K_{OW} between 4 and 8. For these compounds, growth is important, although dietary uptake and biotransformation may substantially impact the BMF; therefore, this is the range of K_{OW} for which *in vivo* testing is generally most important for biomagnification in fish.

Traditional methods for fish bioaccumulation testing are complicated by growth effects as well as the difficulty of maintaining a controlled chemical exposure. Dr. MacLeod described the chemical benchmarking approach to help address these uncertainties^{17, 18}. Using hexachlorobenzene (HCB) as an example, Dr. MacLeod showed a case study in which decreasing concentrations of HCB during the depuration phase of a BCF test were largely due to growth dilution. By benchmarking other substances against HCB it was possible to obtain growth-corrected rate constants for elimination as well as more consistent data. Based on this approach, a proposed “Better BCF” test combines elements of the abbreviated testing protocol referred to earlier with chemical benchmarking. Using five hydrophobic test compounds, Adolfsson-Erici and colleagues¹⁷ obtained good agreement between Better BCF test results and BCFs obtained using the full OECD 305 protocol¹⁴. Finally, Dr. MacLeod described recent efforts to extend the benchmarking approach to obtain information related to dietary uptake and biotransformation. The results of these studies suggest that by using several benchmarking substances, it may be possible to estimate the growth rate, dietary absorption efficiency, and k_B from a single standardized dietary test protocol¹⁹.

Biotransformation: *In Vivo* and *In Silico*. For many years the prediction of k_B was seen as an intractable problem. This situation is now changing. In this session Dr. Arnot showed how *in vivo* k_B can be estimated from laboratory data such as the previously described BCF and BMF testing datasets and Dr. Papa described QSARs for predicting k_B (or the associated biotransformation half-life, HL_B) from chemical structure.

Dr. Arnot began by describing a simple mass balance model that is widely used to predict chemical accumulation in fish. Within the model k_B is one of four terms that contribute to the total elimination rate constant (k_T). Importantly, k_B operates against the whole-body chemical concentration (this becomes relevant when *in vitro* systems derived from liver tissue are used to predict k_B ; see next session). The other three terms that contribute to k_T are the branchial elimination rate constant (k_2), the fecal elimination rate constant (k_E), and the growth rate constant (k_G). Dr. Arnot showed how the mass balance model can be used to estimate k_B from different bioaccumulation measurements²⁰. In essence, the model is used to estimate the k_B that “explains” the difference between an observed BCF and the BCF that would have been predicted in the absence of biotransformation. A similar approach can be used to solve for k_B based on measured values of k_T . Finally, Dr. Arnot described the development of an *in vivo* k_B database²¹, which was generated by modeling measured and evaluated laboratory testing data as summarized in the previous session.

In the second half of the session, Dr. Papa began by noting the growing interest for the use of QSARs in chemical regulation. Although initially driven by a lack of testing data, the QSAR approach is consistent with the goal of reducing animal testing, promoted in recent legislation (e.g., REACH). Quantitative relationships between a chemical’s structure and its behavior in both physical and biological systems reflect

the interactions of molecules with their environment, including other molecules and macromolecules. As such, the approach is based on fundamental principles of chemistry, biology, and physics. Because of their large and growing role in chemical assessment, an effort has been made to develop principles for the acceptable use of QSARs ^{22, 23}. These principles include the identification of a defined endpoint, development of unambiguous algorithms, definition of the model domain of applicability, evaluation of goodness of fit, robustness, and predictive power, and (if possible) a mechanistic interpretation of results.

Dr. Papa presented her results for the development of HL_B -QSARs for fish based on theoretical molecular descriptors ²⁴. She described in general terms the strategy for QSAR model development, including the selection of molecular descriptors and use of existing data to train and “validate” the model. The importance of the applicability domain to correctly evaluate the reliability of predictions and compare different models was also highlighted. Dr. Papa summarized other QSAR approaches and chemical descriptors applied to the *in vivo* k_B database described earlier, along with other data sources, to develop and “validate” different HL_B -QSARs for fish ²⁴⁻²⁷. Dr. Papa described how different individual QSARs can be combined to increase the overall accuracy of modeled predictions ²⁴. Dr. Papa demonstrated that the combinatorial approach, together with an examination of the applicability domain, can increase confidence in using predictions. Additionally this approach is useful to identify i) uncertainties in experimental and predicted data, ii) data gaps, and iii) technical/analytical challenges for new experiments. These examples showed that *in silico* models correctly developed, applied, and combined are powerful tools to screen chemicals.

Biotransformation: In Vitro to In Vivo Extrapolation (IVIVE). Dr. Laue presented the application of the *in vitro*-to-*in vivo* extrapolation (IVIVE) method ²⁸ for estimating biotransformation rates in fish for a number of fragrance compounds ²⁹. Her work showed that if *in vitro*-derived biotransformation information is used in bioaccumulation assessment then model calculated BCFs are in better agreement with measured BCFs for fish than if biotransformation is ignored. This work, as well as several related studies with other compounds (e.g. ³⁰) illustrate that the IVIVE approach is a promising method for improving bioaccumulation assessment of hydrophobic organic substances that can be biotransformed by fish. The on-going inter-laboratory comparison for the IVIVE approach to support OECD test guideline development led by the ILSI Health and Environmental Sciences Institute was also mentioned.

Dr. Nichols discussed scientific challenges that require further investigation and outlined how the IVIVE method can contribute to a WoE approach to bioaccumulation assessment. The scientific issues that require further research include the general under-prediction of the hepatic intrinsic clearance and *in vivo* biotransformation rates; the role of non-hepatic biotransformation; the concentration dependence of the biotransformation rate (as described by Michaelis Menten kinetics); the role of protein binding and allometric scaling of

biotransformation rates. In addition, Dr. Nichols suggested that the “free chemical” hypothesis used as the basis for IVIVE in mammals may be inappropriate for predicting the biotransformation of hydrophobic organic chemicals by fish. Finally, Dr. Nichols pointed out that while there are indications that IVIVE can be a useful addition to bioaccumulation assessment techniques, there is to date a lack of studies aimed at “validating” IVIVE methods for fish and that there is a critical need for studies in which BCFs are generated for the same animals used to obtain *in vitro* data.

In group discussions the participants agreed that the IVIVE approach is a very promising method for bioaccumulation assessment. With continued research focused on addressing current uncertainty and validation of the general methodology, the method can become a valuable addition to the arsenal of bioaccumulation assessment techniques and will contribute to a WoE approach to bioaccumulation assessment. A focused research effort to advance the application of IVIVE in bioaccumulation assessment could hasten the development of practical tools and methodologies to support decision making.

Bioaccumulation Assessment. The following points were discussed in the final summary session and highlight key points raised and discussed at the workshop:

- Current bioaccumulation regulations in most jurisdictions include only the bioconcentration factor (BCF) and the octanol-water partition coefficient (K_{ow}) for screening assessments. Methods for evaluating bioaccumulation continue to evolve and various other metrics have been proposed including the biomagnification factor (BMF), bioaccumulation factor (BAF), trophic magnification factor (TMF), and elimination half-life ($t_{1/2}$)^{1,2}.
- Environmental measurements are most relevant regarding a chemical’s actual behavior in the environment; however, due to natural variability, field measurements, and hence field bioaccumulation data, can be quite variable. Environmental sampling that does not consider variability can translate to uncertainty, which reduces the precision of field-derived bioaccumulation metrics which may be important in a regulatory context. Models and guidance are recommended to develop consistent methods for collecting and interpreting field data.
- The BCF does not account for chemical uptake from the diet; therefore, it may not reflect the extent of bioaccumulation that would result from an environmental exposure. However, models can be used to relate the BCF to environmentally relevant metrics such as the BMF, BAF, and TMF in a straightforward manner³.
- Current science indicates that the biotransformation rate constant (k_B) represents the principal source of uncertainty in the bioaccumulation

assessment of most chemicals with high bioaccumulation potential and k_B is a common element to all bioaccumulation assessment metrics. It is clear that more research to improve the quantification and estimation of biotransformation rates and pathways is critical for improving bioaccumulation screening assessments. *In vivo* k_B databases and *in silico* models (quantitative structure-activity relationships, QSARs) for predicting k_B from chemical structure have recently been established, but more k_B data are needed and a wider range of measurement techniques should be developed. *In vitro* and *in vitro-to-in vivo* extrapolation (IVIVE) methods and data to estimate clearance and k_B continue to evolve and offer a promising path forward. Since k_B is often the key determinant of existing and proposed bioaccumulation assessment metrics, it was suggested that k_B could be used as a new screening-level criterion for bioaccumulation assessment. A combination of estimates for k_B derived from different methods can be applied in a weight of evidence approach (WoE) to address uncertainty.

- A weight of evidence (WoE) is suggested for screening assessments (to the greatest extent possible, often with limited data) and certainly for definitive decision-making (cases in which more data are available or can be obtained). A WoE framework has been developed for biomagnification assessment ¹. A complimentary framework and formal guidance for overall bioaccumulation assessment is suggested.

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Appendix:**Workshop Organizing Committee:**

Name	Affiliation
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Pim Leonards	VU University Amsterdam
Matthew MacLeod	Stockholm University
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Heike Laue	Givaudan Schweiz AG