Case study on the Use of Integrated Approaches for Testing and Assessment (IATA) for Bioaccumulation - Ninth Review Cycle (2023)

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This publication was developed in the IOMC context. The contents do not necessarily reflect the views or stated policies of individual IOMC Participating Organizations.

The Inter-Organisation Programme for the Sound Management of Chemicals (IOMC) was established in 1995 following recommendations made by the 1992 UN Conference on Environment and Development to strengthen co-operation and increase international co-ordination in the field of chemical safety. The Participating Organisations are FAO, ILO, UNDP, UNEP, UNIDO, UNITAR, WHO, World Bank, Basel, Rotterdam and Stockholm Conventions and OECD. The purpose of the IOMC is to promote co-ordination of the policies and activities pursued by the Participating Organisations, jointly or separately, to achieve the sound management of chemicals in relation to human health and the environment.

Foreword

OECD member countries have been making efforts to expand the use of alternative methods in assessing chemicals. The OECD has been developing guidance documents and tools for the use of alternative methods such as (Q)SAR, chemical categories and Adverse Outcome Pathways (AOPs) as a part of Integrated Approaches for Testing and Assessment (IATA). There is a need for the investigation of the practical applicability of these methods/tools for different aspects of regulatory decision-making, and to build upon case studies and assessment experience across jurisdictions.

The objective of the IATA Case Studies Project is to increase experience with the use of IATA by developing case studies, which constitute examples of predictions that are fit for regulatory use. The aim is to create common understanding of using novel methodologies and the generation of considerations/guidance stemming from these case studies.

This case study was developed by Health and Environmental Sciences Institute (HESI), Canada, United Kingdom, United States, Business at OECD (BIAC) for illustrating practical use of IATA and submitted to the 2023 review cycle of the IATA Case Studies Project. This case study was reviewed by the project team.

This case study is an illustrative example, and its publication as an OECD monograph does not translate into direct acceptance of the methodologies for regulatory purposes across OECD countries. In addition, this case study should not be interpreted as official regulatory decisions made by the authoring member countries.

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Executive Summary

Bioaccumulation is the net result of competing rates of chemical uptake into, and elimination from, an organism. Bioaccumulation assessment is an essential endpoint in national and international chemical regulatory programmes and treaties, e.g., the identification of Persistent, Bioaccumulative and Toxic (PBT) substances and Persistent Organic Pollutants (POPs). Bioaccumulation assessment is both a scientific and regulatory challenge due to the intricacy of the subject, the availability of reliable and relevant data, and lack of guidance in how data should be evaluated and weighted to inform decision-making. This process becomes more challenging considering variations in accepted data and the varying threshold criteria of different regulatory jurisdictions. A weight of evidence (WoE) approach is required by most established regulatory jurisdictions, such as the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) program in Europe, the Canadian Environmental Protection Act (CEPA), and the United Nations Stockholm Convention; however, there is no clear guidance for implementing a WoE approach for B assessment in these programmes. Multiple lines of evidence (LoE) can be used to quantify, measure, and qualify the common metrics used in B assessment; namely the bioconcentration factor (BCF), bioaccumulation factor (BAF), biomagnification factor (BMF) and trophic magnification factor (TMF). These LoE can comprise *in silico* predictions, combinations of *in vitro* and *in silico* data, e.g., *in vitro-in vivo* extrapolation (IVIVE), and measured laboratory or field data. Quantitative Structure-Activity Relationship (QSAR) models for BCF and BAF, and biotransformation half-lives, as well as mechanistic toxicokinetic (TK) models for calculating B assessment metrics are established. Additionally, there are Organisation for Economic Cooperation and Development Test Guidelines (OECD TG) for the laboratory determination of *in vitro* fish biotransformation rates, BCFs, and BMFs.

This Integrated Approach for Testing and Assessment (IATA) for Bioaccumulation encompasses the LoE outlined above and existing OECD TG and WoE principles. This IATA provides examples to aid evaluators in the collection, generation, evaluation, and integration of multiple LoE for clear and transparent decision-making within defined problem contexts. This IATA includes guidance for data collection and generation from publicly available databases and models that can be readily used for data poor and relatively data rich chemicals. The data evaluation criteria within the IATA are primarily developed from existing OECD TG for each LoE (e.g., OECD TG 319 A/B), providing a systematic approach to address uncertainty within each individual LoE. As each LoE is evaluated, the guidance and methods in this IATA provide a systematic, pragmatic, iterative, and transparent process to determine if there is sufficient confidence in the available data and/or WoE approach for decision-making. Three illustrative case studies representing both data poor and data rich chemicals are presented to illustrate the applicability of the IATA for Bioaccumulation assessment.

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1 Introduction

Bioaccumulation is the net result of competing rates of chemical uptake into, and elimination from, an organism. Chemicals are subject to bioaccumulation ("B") assessment as part of national and international regulatory programs and treaties (Government of Canada 1999; UNEP 2001; 2019; EC 2003a, 2003b; European Parliament 2006; UK Government 2021). This assessment can be both a scientific and regulatory challenge due to the various metrics for assessing bioaccumulation in aquatic and terrestrial organisms and food webs, diverse regulatory criteria, and variability and uncertainty in bioaccumulation data. For the purposes of B assessment, multiple lines of evidence (LoE) can be used to quantify, measure, and qualify the common B metrics. **Table 1** lists definitions and provides an overview of B metrics considered in this IATA. These include the bioconcentration factor (BCF), bioaccumulation factor (BAF), biomagnification factor (BMF) and trophic magnification factor (TMF). These LoE can be obtained from laboratory or field measurements, from *in silico* (model) calculations, or from a combination of *in vitro* measurements and *in silico* predictive calculations. Traditionally, B assessment has focused on aquatic organisms and ecosystems only, as this was considered protective of both aquatic and terrestrial organisms; however, research has shown the fundamental differences in bioaccumulation potential between water-ventilating and air-breathing species (Gobas 2003; Czub 2004; Armitage 2007; Kelly 2007; OECD 2018c), and LoE and criteria for air-breathing organisms are being considered (Arnot 2022; Wania 2022; Saunders 2023b).

Table 1. Overview of B metrics considered in this IATA

Metric *[data type]*	Definition	Species / taxa	Model systems
BCF Bioconcentration Factor *[lab]*	The ratio of the steady-state chemical concentrations in a water-respiring organism to the **water.**	Fish	*In silico* *In vivo*
		Invertebrate	*In silico* *In vivo*
BMF Biomagnification Factor *[lab or field]*	The ratio of the steady-state chemical concentrations (or fugacities or activities) in an organism **compared to its diet.**	Fish	*In silico* *In vivo*
		Rat	*In silico* *In vivo*
		Various	*In silico* *In vivo*
BAF Bioaccumulation Factor *[field]*	The ratio of the steady-state chemical concentration in an organism to the water where it resides including exposure to chemical from its **surrounding environment and its diet**.	Fish	*In silico* *In vivo*
		Invertebrate	*In silico* *In vivo*
		Various	*In silico* *In vivo*
TMF Trophic Magnification Factor *[field]*	The average factor across an entire food web by which the chemical concentrations (or fugacities or activities) in biota change **per trophic level within a food web.**	Various	*In vivo*

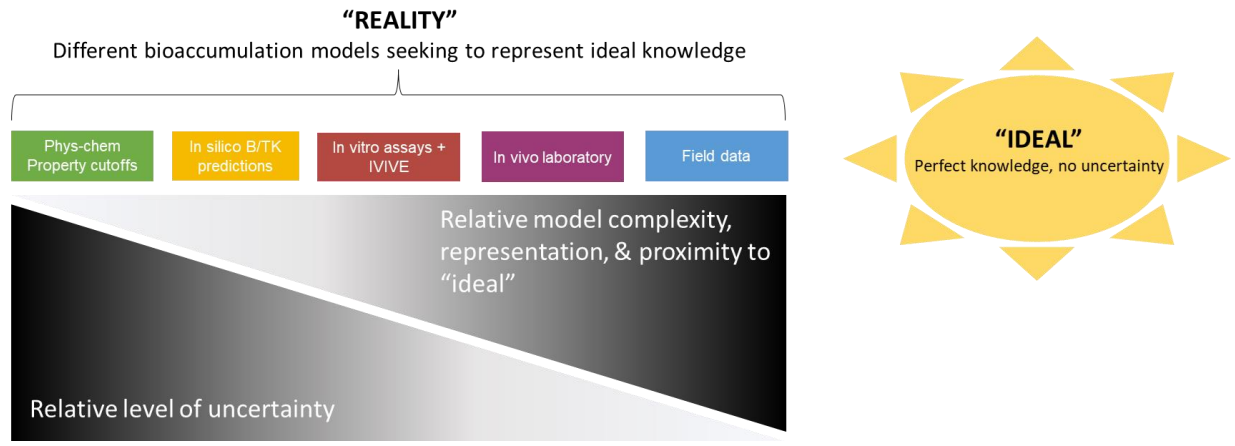
Note: Field refers to the natural environmental conditions and lab refers to controlled experimental conditions. *In silico* refers to model calculations and *in vivo* refers to measured, empirical values under these conditions in organisms.
Source: (Arnot 2006; Gobas 2009; Burkhard 2012; Arnot 2023)

The octanol-water (K_{OW}) and the octanol-air (K_{OA}) partition ratios are basic physical-chemical properties used for B screening assessment (Mackay 1982; Arnot 2022). Quantitative Structure-Activity Relationships (QSARs) and Poly-Parameter Linear Free Energy Relationships (PPLFERs) and other methods can be used to predict physical-chemical properties and biotransformation half-lives in the absence of measured values or to corroborate measured data. Predicted chemical information is routine to address data gaps in chemical assessments. For example, in the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) Regulation in Europe chemicals with log K_{OW} > 4.5 are screened in for B assessment. QSARs are also employed for predicting relevant B assessment data, e.g., fish BCFs (Dimitrov 2002; Dimitrov 2003; Dimitrov 2005; Fernandez 2012; Gissi 2015; Petoumenou 2015) and BAFs (Arnot 2003; Costanza 2012) and for biotransformation half-lives (Arnot 2009; Brown 2012; Arnot 2014; Papa 2014; Papa 2018). Standardized testing methods for physical-chemical properties (OECD 2023a) and fish BCF and BMF experiments (OECD 2012) have been developed by the Organisation for Economic Cooperation and Development (OECD). Furthermore, databases of measured fish BCFs and BMFs have been established and in some cases subject to data quality (reliability) assessments (Arnot 2006; Arnot 2015). In addition, mass balance physiologically-based biokinetic (PBK) models have successfully been developed to quantify the bioaccumulation of organic chemicals in aquatic and terrestrial wildlife and humans, e.g., (Gobas 2003; Smítková 2005; Armitage 2007; Armitage 2013; Goss 2013; Wu 2022). These organism-specific bioaccumulation models simulate chemical-specific uptake and elimination processes within an organism and form the basis for mass balance food web bioaccumulation models. In summary, there are various measured and predicted data that can be relevant to inform B assessment decision-making.

However, sometimes available data can provide contrasting results from multiple LoE, which can result in conflicting “B” conclusions and in such instances a Weight of Evidence (WoE) approach may be required.

Ideally, perfect knowledge relating to the bioaccumulation of organic chemicals in the biosphere would be available; however, this is not the reality under which scientific and regulatory decisions must be made. **Figure 1** provides a conceptual overview of different models that can be used in efforts to determine bioaccumulation to inform decision-making. All LoE are models seeking to represent the complex and dynamic conditions of the real-world. For example, field measurements are generally considered to be the most representative data for evaluating bioaccumulation; however, field data are snapshots in space and time and without exhaustive and impractical sampling requirements are uncertain and incomplete (i.e., one cannot sample all species in all ecosystems for B assessment). Laboratory test methods and select species are also models for the bioaccumulation processes that occur in multiple and variable species and ecosystems. In terms of scientific relevance, the field data are the most representative of ideal knowledge and the physical-chemical screening criteria are the most simple and uncertain LoE for representing the complex reality of bioaccumulation. The physical-chemical properties are based on relationships with bioaccumulation metrics and historical knowledge of bioaccumulation, but they do not include biological, toxicokinetic processes that also influence bioaccumulation.

Figure 1. Conceptual overview of different models that can be used in efforts to determine bioaccumulation to inform decision-making



This IATA presents a method for performing a bioaccumulation assessment following a weight of evidence (WoE) approach for discrete organic chemicals in both aquatic and terrestrial environments to support various decision contexts. This WoE approach provides a consistent, yet iterative data integration framework for decision-making that is transparent and logical and generally follows the “Guiding Principles and Key Elements for Establishing a Weight of Evidence for Chemical Assessment” developed by the OECD (OECD 2019). A WoE approach is required in most regulatory programs (European Parliament 2006; US EPA 2017); however, there is often no clear implementation guidance and/or WoE strategy, making it difficult for stakeholders to collect, generate, integrate, evaluate, and compare various LoE for ‘B’ assessment decision-making. A WoE approach for B assessment following OECD guidance has been developed (Arnot 2023) and these methods have been formalized into the Bioaccumulation Assessment Tool (BAT) and its user manual (ARC Arnot Research and Consulting 2019). The approach is incorporated

into the BAT to operationalize the methods and guidance as a user-friendly tool, which is a freely-accessible online resource (www.arnotresearch.com/BAT).

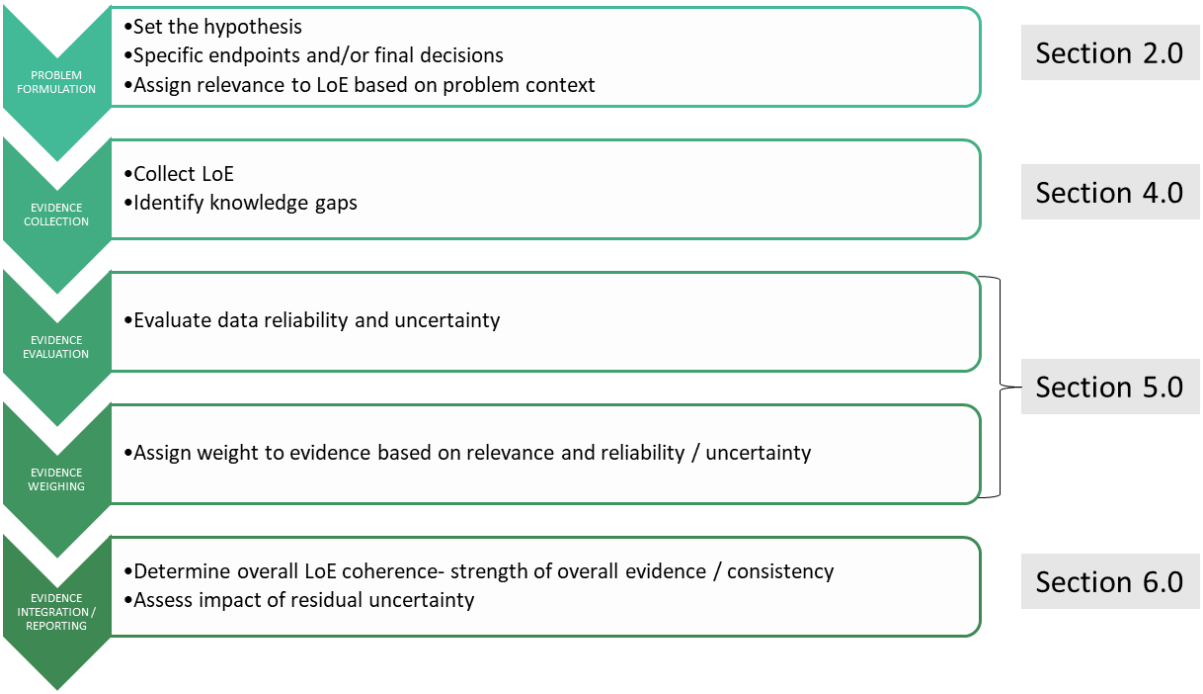
Briefly, the B assessment guidance and the BAT facilitate the collection, generation, evaluation, and integration of multiple data sources and LoE to inform decision-making across various B assessment contexts for aquatic and air-breathing organisms. The guidance and the BAT consider *in vitro*, *in silico*, *in vivo* and field data. The BAT guides the user to enter and evaluate various measured LoE (e.g., **Table 1**) and includes mass balance bioaccumulation models for representative aquatic and terrestrial organisms and food webs. The bioaccumulation models in the BAT are useful for addressing data gaps and for data-poor chemicals (i.e., those with no or limited measured LoE). The same bioaccumulation models in the BAT can also be used independently in a more readily applicable on-line tool known as the Bioaccumulation Estimation Tool (BET). The BET and BAT include various mass balance bioaccumulation (toxicokinetic) models for aquatic (water-respiring) and terrestrial (air-breathing) organisms and food webs under laboratory and environmental conditions. The BET models are built into the BAT so one can proceed from screening-level B metric estimates for "data poor" chemicals (i.e., no, or very limited measured data for B assessment) to a WoE approach including other LoE, i.e., measured B metrics from the lab and field and other *in silico* B metric predictions. Other publicly available tools and models for predicting bioaccumulation data can also be considered in this IATA and in the WoE approach implemented in the BAT.

The BET is implemented in the freely-accessible on-line Exposure And Safety Estimation (EAS-E) Suite platform (www.eas-e-suite.com). As implemented in EAS-E Suite, the BET only requires chemical name, Chemical Abstract Service Number (CAS), or Simplified Molecular Input Line Entry System (SMILES) notation as user-input to obtain bioaccumulation model predictions for representative aquatic and air-breathing species. The BET initially provides the required model input parameters (e.g., K_{OW}, K_{OA}, biotransformation half-lives) using built-in databases, QSARs and PPLFERs. Reliable measured data are selected preferentially over model predictions to parameterize the BET. However, the BET provides opportunities for users to revise the initial parameters with preferred values (e.g., *in vivo* biotransformation half-lives not included in the built-in databases). EAS-E Suite also includes *in vitro-in vivo* extrapolation (IVIVE) models so that *in vitro* biotransformation rates can be used to parameterize the BET. Furthermore, the BET can be parameterized with measured or predicted biopartitioning data such as membrane-water and protein-water partition ratios to replace octanol-based biopartitioning assumptions, if desired. For Ionizable Organic Chemicals (IOCs) users are required to obtain chemical dissociation values (i.e., pKas).

A WoE approach is a process of assembling, evaluating, weighing, and integrating evidence to come to a scientifically defensible conclusion that can be used when multiple LoE are available. A WoE approach should provide a consistent framework for decision-making and needs to be transparent. The OECD recently formalized recommendations for a WoE approach for chemical evaluations (OECD 2019). This document outlines the following key elements in a WoE approach: (i) problem formulation (establish hypothesis, criteria, and metrics), (ii) evidence collection, (iii) evidence evaluation, (iv) evidence weighing, and (v) evidence integration/reporting (OECD 2019). **Figure 2** highlights key considerations in the OECD WoE guidance (OECD 2019) that are part of this IATA. Notably, the recommended approach highlights that data reliability and uncertainty must be addressed. Methods and criteria for evaluating reliability have been developed for various data types and decision contexts. For example, Klimisch data reliability scoring methods developed for ecotoxicity data (Klimisch et al., 1997) are considered broadly. Data reliability criteria for ecotoxicity and fate studies supporting the US Environmental Protection Agency's Toxic Substances Control Act (TSCA) existing chemicals risk evaluations are detailed in the EPA's draft systematic review protocol supporting TSCA risk evaluations for chemical substances (US EPA 2021). The

data reliability (quality) assessment methods and criteria outlined in this IATA were explicitly developed for B assessment and to the greatest extent possible following OECD Testing Guidance for most LoE.

Figure 2. Key WoE elements considered in this Bioaccumulation IATA



Source: modified from (OECD 2019)

This B IATA provides the tools to transparently evaluate all data consistently and objectively using a WoE approach and identify uncertainties and gaps that can be addressed in a systematic manner (i.e., an integrated approach to testing) to foster confidence in the decisions. The framework can be used in a tiered manner but is intended to be iterative and efficient. The broadly applicable B IATA provides the flexibility within the recommended tools to adhere to the data requirements and thresholds of different regulatory jurisdictions and should prevent duplication of work. Due to the diversity in models, metrics, and testing approaches (e.g., BCF, BAF, BMF, TMF), this IATA facilitates the generation, collection, data quality evaluation and integration of varied data types that users can further consider in a WoE approach.

This IATA does not consider laboratory or field derived biota-soil accumulation factors or biota-sediment accumulation factors (BSAFs), such as those derived using the OECD TG 315 (*Lumbriculus*; (OECD 2008)) or OECD TG 317 (Terrestrial Oligochaetes; (OECD 2010a)). The BSAF studies are often only performed when specific concerns around exposure pathways have been identified and therefore it is at the discretion of an evaluator to decide at which stage these data should be included in an assessment. For example, during exposure assessment, BSAF data are useful for calculating internal tissue concentrations to compare to toxicological benchmark values for soil or sediment invertebrates. Sediment invertebrates can be a key source of trophic transfer of contaminants from the benthos to higher trophic level animals for persistent chemicals and bioaccumulation in soil and sediment invertebrates can be included in the IATA if measured TMFs with such species are available. Field BAFs and field BMFs are also calculated for benthic and aquatic invertebrates in the BET and BAT models.

Wildlife biomonitoring data provide evidence of environmental occurrence and thus exposure concerns. However, detection of a substance within organisms alone is not indicative of bioaccumulation and additional scrutiny must be applied. Bioaccumulation hazard metrics require a reference of exposure, e.g., concentration in diet or the environment. This issue is discussed further in the Environment Agency, UK's "Guidance on interpreting biota tissue concentrations for bioaccumulation assessment" (Environment Agency 2022) and thus mere detection of a chemical in an organism is not considered in this IATA. Of course, the detection of a chemical in biota could provide the impetus for a B assessment for which this IATA is intended.

2 Purpose and Hypotheses (Problem Formulation)

The purpose of any IATA is to gather available evidence to support or reject one or more hypotheses (questions) formulated to support the solution to an assessment or research problem. The first step in the assessment process is to define the problem which can be posed as a question or a hypothesis for which data can be used to answer the question. In other words, asking the questions “what is the problem?” and “what data do I need to help solve it?”

This *problem formulation* step also involves identification of purpose / context for which a B assessment is being performed. This can include, regulatory prioritization and screening exercises; United Nations Globally Harmonized System of Classification and Labelling of Chemicals (GHS); registration of a substance, e.g. EU and UK REACH (European Commission 2007; UK Government 2021) require a persistent, bioaccumulative, and toxic (PBT) assessment for those manufactured or imported in quantities equal to or more than 10 tonnes per year (Section 4 Annex 1 and Annex 13 of REACH regs; (European Commission 2007, 2011)); substances that have been prioritized for evaluation that are suspected to be PBT/vPvB (Substances of Very High Concern); PBT/vPvB substances that have the potential for long range transport (Persistent Organic Pollutants). The decision context is critical for understanding the acceptable or tolerable level of uncertainty. For example, a higher level of uncertainty may be tolerated during prioritization and screening exercises where a substance is concluded to be ‘not B’, or ‘potentially B/vB’. These stages are associated with a higher acceptance of false positives thereby reducing false negative outcomes. Depending on the outcome this may require the generation of further data to address gaps or uncertainties. In contrast, there is a low tolerance for uncertainty, where the output from this IATA is used as a conclusion on bioaccumulation (e.g., PBT assessments, SVHC determinations or Stockholm Convention POP nominations).

This B IATA is intended to guide the collection, generation, evaluation, and weighing of various types of bioaccumulation data including physical-chemical, *in silico*, *in vitro* and *in vivo* data. Central to this IATA are the elements and principles of WoE such as those outlined in OECD (OECD 2019), most notably problem formulation and data relevance and reliability. Bioaccumulation data have been used to help answer various questions in varying regulatory and non-regulatory contexts (e.g., agency vs national vs territorial vs global contexts). Below are some example problem statements that may be appropriate for the decision/assessment contexts:

- Is there sufficient evidence to conclude that a substance meets a regulatory BCF and/or BAF criteria of greater than or equal to a given threshold (i.e., 5000 L/kg-ww)? See **Table 2.**
- Is there sufficient evidence to conclude that a substance also has a BMF and/or TMF > 1? (e.g., hazard assessment, monitoring, and compliance programs).

To illustrate the discussion, example case studies are given in **Section 7** that include problem formulation considerations for two data poor scenarios and one data rich scenario.

2.1 Application

Chemicals are subject to B assessment as part of national and international regulatory programs and treaties (Government of Canada 1999; UNEP 2001; 2019; EC 2003a, 2003b; European Parliament 2006; ECHA 2017a). A summary of the various criteria used by different regulatory jurisdictions is provided in **Table 2**. A table of references for jurisdiction, legislation and where relevant, guidance texts, is presented in **Annex B. Table A B.1.**

Table 2. Examples of aquatic bioaccumulation thresholds and endpoints defined in law and guidance of different jurisdictions

Jurisdiction	Criteria	
	Screening	**Definitive***
European Union	log K_{OW} ≥ 4.5 (potentially B)	BCF ≥ 2000 (B)
United Kingdom	log K_{OW} ≥ 5 (potentially vB)	BCF ≥ 5000 (vB)
United States		BCF 1000 – 5000 (B)
		BCF ≥ 5000 (highly B)
Canada		BAF ≥ 5000 (B)
		BCF ≥ 5000 (B)
		log K_{OW} ≥ 5.0
Japan	log K_{OW} ≤ 3.5 or a molecular weight ≥ 800 (≥ 1000 for halogens)	BCF < 1000 (not highly B)
		BCF 1000 – 5000 (B) (expert judgement required)
		BCF ≥ 5000 (highly B)
Australia		log K_{OW} ≥ 4.2 (B, if BCF/BAF not available)
		BAF and/or BCF ≥ 2000 (B)
		BMF > 1 (B)
United Nations	log K_{OW} ≥ 5.0	BCF or BAF ≥ 5000

*Units of BCF and BAF are L-water/kg-fish wet weight

Terrestrial bioaccumulation assessment is not performed widely. However, the currently proposed chemical property thresholds to screen for bioaccumulation potential in air-breathing organisms are log K_{OW} > 2 and log K_{OA} > 5. These criteria are employed in EU and UK REACH (European Commission 2007; UK Government 2021). Additionally, ECHA has produced a discussion paper on the bioaccumulation assessment of air-breathing mammals. The Australian guidance indicates that a conclusion for bioaccumulation in the terrestrial compartment can be made if a substance has a log K_{OW} ≥ 2 and a log K_{OA} > 6 (Australian Government 2020).

Guidance texts associated with performing a B assessment exist for 5 of the jurisdictions noted in **Table 2 and Annex B, Table A B.1.** and are mainly related to the identification of PBT substances. For the B endpoint, the level of detail varies from re-iterating the relevant threshold criteria to a described testing strategy. However, these guidance texts are only relevant to the authoring jurisdiction.

This IATA is intended to be used where a B assessment must or should be performed for chemicals in both aquatic and/or terrestrial systems. Its use should be considered for various decision or assessment contexts including, but not limited to:

- Classification & labeling
- Prioritization exercises for chemicals of concern
- Hazard assessment
- Identification of testing gaps / needs (e.g., characterization of uncertainties & identification of needed data to increase confidence)
- Supporting a testing proposal (e.g., development of a tiered approach)

This IATA illustrates an approach that could be used on discrete organic chemicals. Three chemicals are used to demonstrate the application of this IATA in **Section 7.** These chemicals were selected for demonstrative purposes only and do not represent the decisions of any specific regulatory authority, nor are they intended to refute existing regulatory assessments for the same chemical. It should be noted that inclusion of different data sources may lead to a different outcome; however, this IATA provides a transparent and repeatable process to understand the approach and methods that underpin any assessment.

This IATA could also be applied within the broader context of risk assessment when using B metrics to convert external environmental concentrations to internal concentrations for subsequent comparisons with toxicity data. However, we do not discuss this application in detail within this current IATA.

It should be noted that the application of this IATA is not always about determining data sufficiency and evidence strength for specific decision contexts. It can be used in situations where no decision is required and is instead used as an integrated approach for information gathering, organization and examination for learning and training purposes, informing monitoring programs, determining vulnerable or most exposed species, posing questions for further research, or examining the relationship between bioaccumulation and toxicity, i.e., toxicokinetics.

As shown in **Table 2**, there are various threshold values for “B” and “vB” across regulatory jurisdictions. Use and application of this IATA should start with the user selecting which threshold values they intend to follow for the purpose of the assessment.

2.2 Target Chemicals

This IATA can be applied to discrete organic chemicals including discrete organic chemical structures selected to represent known structural components of unknown or variable composition, complex reaction products, or biological materials (UVCBs). Metals, polymers, UVCBs, nanoparticles, and microplastics are not included in this IATA. Most *in silico* models have been developed based on the bioaccumulation knowledge generated for individual neutral organic substances and often within a specific range of log K_{OW} (e.g., 1-6). Within the last ten years, methods have been developed to address the biopartitioning of ionogenic substances (e.g., pKa range 2-10) within *in silico* tools (Armitage 2013; Kierkegaard 2021) useful for such chemical classes as some perfluoroalkyl substances (PFAS) and amine surfactants. Modifications to testing methods (e.g., OECD Test Guidelines) have existed for ionizing substances for some time (e.g., pH buffer testing) and have been used for chemicals such as triclosan.

2.3 Endpoint(s)

Table 1 provides an overview of the bioaccumulation endpoints ("B metrics") used in this IATA and includes B metrics in aquatic and terrestrial systems which can vary depending on the jurisdiction (see **Table 2**) and the problem statement. For example, if the question posed is "what is the bioconcentration factor in fish", the endpoint of interest is the BCF.

2.4 Identifying Relevant Lines of Evidence (LoE)

Within the problem formulation step of this IATA, the user must indicate their subjective relevance score for each B assessment metric. Although subjective, there are guidelines available that can aid users for this step (e.g., OECD 2019). In OECD (2019) the term "relevance" describes whether a procedure is meaningful and useful for a particular purpose (i.e., "fit for purpose"). In this Bioaccumulation IATA, the term relevance thus refers to the appropriateness or degree of correspondence of evidence to the question(s) outlined in the problem formulation. Fit for purpose is often context dependent, meaning the weight given to relevance will vary according to the level of confidence required to address the purpose. For example, a lower tier B assessment may not require the degree of confidence a higher tier assessment would if further investigation is triggered at the lower tier. Conversely, a lower tier assessment may require high accuracy if the decision context results in "no further action" (i.e., an off ramp) and a high level of confidence is required to justify this conclusion. The definition of relevance provided in Hall (Hall 2017) is also useful because it refers to both the regulatory and scientific relevance (biological and exposure). For example, using K_{OW} alone to describe bioaccumulation in aquatic receptors generally has lower regulatory and scientific relevance compared with *in vivo* laboratory BCF data. The BAF has very high relevance in Canada and for Stockholm Convention POP assessments because it is a field metric of bioaccumulation. While explicit mention of the BAF does not exist in some regulatory programs it can still be considered, e.g., US EPA considers BAFs as well as BCFs in fish (Costanza 2012). Guidance for seeking and gathering LoE deemed relevant by an evaluator is provided in **Section 4**. The relative relevance applied to each LoE is context and evaluator specific. This IATA and the BAT provide flexibility (user discretion) for assigning the relative relevance for each LoE. It is important that relative relevance for each LoE is determined *a priori* during the problem formulation stage; however, one can "re-set" the relevance of various LoE at their discretion if the relevance is transparently communicated in the process.

To accommodate a means of weighing relevance for a decision context, the case studies presented in this IATA use a simple and relative quantitative scoring scheme of zero to five where lower scores indicate a low degree of regulatory or scientific relevance to the hypothesis using the fit for purpose concept mentioned above. These scores are determined at the discretion of the evaluator (e.g., dependent on jurisdiction policies) but are documented for the sake of transparency when interpreting and communicating the methods and decision points in the decision-making process.

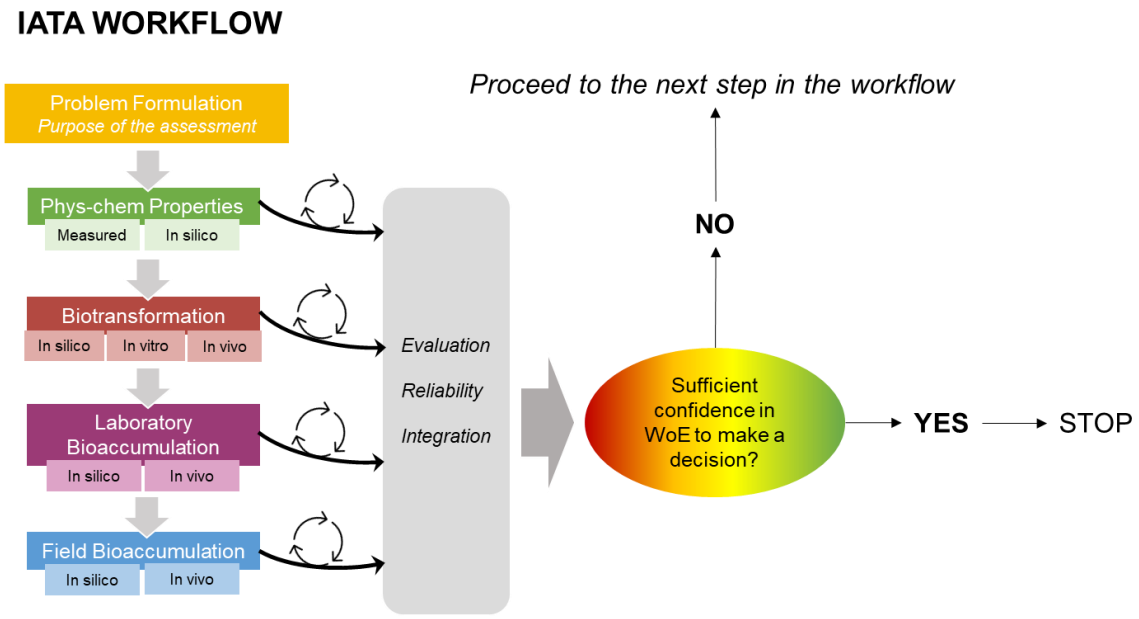
3 Approaches Used

3.1 General Workflow

The B IATA uses a workflow approach whereby a series of steps is performed to gather information, evaluate its reliability and uncertainty, assign a weight to each LoE, and report outcomes. The IATA includes an evaluation of the strength of evidence (SoE) and residual uncertainty (i.e., uncertainty that is not or cannot be accounted for during data evaluation). This workflow can include model predictions and measured data, depending on the problem formulation and the availability of information. Based on the context, the user can make the determination of whether their objectives have been met at various stages or decision points within the workflow and whether additional information needs to be gathered to provide sufficient evidence to support the decision context. In other words, this B IATA facilitates a scientific process that provides information to support a decision. Those decision contexts may be different depending on the associated level of confidence required for the specific context.

In **Figure 3**, a high-level overview of the approach and information used to inform the B IATA is shown. The initial step (problem formulation) identifies the purpose for which the assessment is being performed (**Section 2**), followed by the evaluation of data relevant to each LoE (**Section 4**). Not all LoE may be required for the overall assessment as per the problem formulation (**Section 2**). Prior to integrating and developing an initial conclusion for each LoE, it is important to examine the relevance and reliability of all data and identify any gaps. As each LoE is completed and integrated into the overall assessment, a clear picture of whether there is sufficient confidence in the WoE will emerge and a conclusion can then be made, e.g., B or not B or more data are required. If more data are required, the IATA provides practical guidance for subsequent testing progressing from relatively lower cost animal free methods to more comprehensive and animal intensive data.

Figure 3. A high-level overview of the approach and information used to inform the B IATA



3.2 Theoretical Considerations

The conceptual model for bioaccumulation is well-established. Organisms are exposed to chemicals from their surrounding environment (e.g., air or water) and their diet (e.g., food and water). After contact there can be chemical absorption into the body past an epithelial barrier at a portal of entry, e.g., dermal, intestinal, lung or gill. There can be differences in the amount of chemical absorbed depending on the route of exposure contact (e.g., in water or diet or air), the exposure media, the organism, and the properties of the chemical. Once absorbed the chemical can be distributed to various compartments of the organism (e.g., blood, liver, kidney, muscle, adipose). The chemical can also be eliminated from the body. These basic concepts are also applied in toxicokinetics (TK), and Absorption Distribution Metabolism Excretion (ADME) principles used in the pharmaceutical and veterinary sciences for several decades. The primary difference in applying these concepts to B assessment is that B metrics are calculated by comparing internal exposures (i.e., whole-body concentrations) to an external environmental source of exposure (e.g., water for BCFs and BAFs, and food for BMFs) (OECD 2012).

Bioaccumulation is evaluated at a whole-body level, i.e., OECD 305 BCFs and BMFs in fish (OECD 2012). Thus, a one-compartment model is sufficient for defining the key terms and parameters used routinely in B assessment and in this IATA. The concepts of competing rates of chemical uptake into and elimination from an organism can be expressed mathematically with the following one compartment general bioaccumulation model for a fish:

$$dC_B/dt = k_1 C_{WD} + k_D C_D - (k_2 + k_E + k_R + k_B + k_G) C_B \qquad \text{(Equation 1)}$$

where dC_B/dt is the net change in concentration in the organism (mg/kg) over time t (h), C_B is the chemical concentration in the organism, k_1 is the respiration uptake rate constant $(L(kg{\cdot}h)^{-1})$, C_{WD} is the dissolved concentration in water $(mg{\cdot}L^{-1})$, k_D is the ingestion (dietary) uptake rate constant $(kg(kg{\cdot}h)^{-1})$, and C_D is the

chemical concentration (mg/kg) ingested (diet). The rate constants (h^{-1}) corresponding to chemical elimination via respiratory elimination, fecal egestion, renal excretion, biotransformation, and growth dilution are k_2, k_E, k_R, k_B, k_G, respectively. Growth dilution is a "pseudo" elimination process in that the chemical is not actually eliminated from the organism, but the change in concentration of the chemical is a function of changes in biomass. The first-order total (terminal) chemical elimination rate is the sum of various individual elimination processes, i.e., $k_T = k_2 + k_E + k_R + k_B + k_G$. Since these are first-order rate constants their corresponding half-lives are easily calculated. For example, the whole-body total (terminal) elimination half-life (HL_T) is $\ln 2/k_T$ and the whole-body biotransformation half-life (HL_B) is $\ln 2/k_B$. Physiological parameters relating to organism-specific rates of respiration (from air or water), ingestion (of food or water), and growth are available to derive organism-specific mass balance bioaccumulation models including those for air-breathing organisms, e.g., (Kelly 2007; Arnot 2008a; Powell 2009; Arnot 2014).

At steady-state ($dC_B/dt = 0$), Equation 1 can be simplified to:

$$k_1 C_W + k_D C_D = (k_2 + k_E + k_R + k_B + k_G) C_B = k_T C_B \quad \text{(Equation 2)}$$

where the left side of equation quantifies chemical uptake into the organism through exposures to chemical in food and water (or air for air-breathing organisms) and the right side of the equation quantifies parent chemical elimination from the organism. For fish, chemical elimination via the kidney to water is generally considered inconsequential compared to chemical elimination from the gill to water (much higher exchange rates for the latter) for most chemicals, and thus renal clearance can often be ignored in fish and aquatic (water-respiring) organisms. However, there is a notable exception (Consoer 2014). These same conceptual and mathematical models are used to determine B metrics like the BCF, BMF and k_T from experimental *in vivo* measurements as outlined in standardized guidelines like the OECD 305 Bioaccumulation Testing Guidance (OECD 2012). For example, following OECD 305 TG the BMF can be calculated with the following (kinetic) expression (OECD 2012):

$$BMF_K = k_D/k_T = k_D/(k_2 + k_E + k_R + k_B + k_G) \quad \text{(Equation 3)}$$

4 Data Gathering

Table 3 lists the specific LoE that can be used in this B IATA with additional details on the individual LoE and the evaluation of each LoE provided in the sub-sections below. It is emphasized that the data gathering process in this IATA proceeds from obtaining data that are more readily available (e.g., QSAR predictions) to data that are more resource intensive to obtain, e.g., OECD 305 (OECD 2012), and for most chemicals ("data poor") many of the more resource intensive LoE will not be readily available. The iterative aspect of the IATA allows one to start with existing data and obtain and evaluate more data as warranted by the problem formulation and the desired level of confidence in the results whether using individual LoE or a WoE approach.

Table 3. Lines of Evidence (LoE) and data gathering that can be considered in the IATA

Taxa	Data type(s)	Parameter / metric	IATA Section
Aquatic & terrestrial	Phys-Chem (measured & *in silico*)	Molecular weight	4.1
		K_{OW}	
		K_{OA} (terrestrial homeotherms only)	
		Water solubility	
		Vapour pressure	
		Henry's Law Constant	
		Air-water partition ratio (K_{AW})	
		Solubility in octanol (S_O)	
		Biopartitioning parameters	
		pKa (IOC* only)	4.2
	Biotransformation	*In vivo* biotransformation and elimination rate constants and half-life data	4.3.1
		In silico biotransformation and elimination rate constants and half-life data	4.3.2
		In vitro biotransformation and elimination rate constants and half-life data	4.3.3
		In vitro – in vivo extrapolation	4.3.4
		Biotransformation in additional species / taxa	4.3.5
	Bioaccumulation	*In silico* bioaccumulation models	4.4.1
		Laboratory BCF data	4.4.2
		Laboratory BMF data (Aquatic)	4.4.3
		Laboratory TK and BMF data (Mammals)	4.4.4
		Field BAF	4.4.5
		Field BMF	4.4.6
		Field TMF	4.4.7

*Ionizable organic chemicals (IOC)
Note that not all data listed here are required for using the IATA

4.1 Physical-Chemical Properties – Neutral Organic Chemical

To utilize this IATA, one must obtain at least the following information on the properties of the neutral organic chemical: molecular weight (MW, g/mol) and K_{OW} (and K_{OA} for air-breathing organisms). The property data can be measured (preferred) (e.g., OECD QSAR Toolbox, eChemPortal: https://www.echemportal.org/) or predicted values from property estimation software such as EPI Suite™ (US EPA 2011) and other sources (e.g., US EPA Chemistry Dashboard: https://comptox.epa.gov/dashboard/, EAS-E Suite: www.eas-e-suite.com;). If available, it is also possible to consider water solubility (S_W, mg/L), vapour pressure (VP, Pa), Henry's Law Constant (H, Pa.m^3/mol) or the air-water partition ratio (K_{AW}), and solubility in octanol (S_O, mol/m^3) to seek thermodynamic consistency in the physical-chemical properties. Evaluators are strongly encouraged to critically evaluate measured and predicted physical-chemical property data before they are used. Methods and tools for evaluating property data and obtaining internally consistent solubility and partitioning properties are available, e.g., (Beyer 2002; Schenker 2005; Li 2022).

It is recognized that octanol is not a reasonable surrogate for estimating biological partitioning for some organic chemicals, e.g., ionizable surfactants, surface active substances, quaternary compounds, and some pigments and dyes (Sijm 1999; Hermens 2013; Torralba-Sanchez 2023). For example, for some chemicals measured and predicted storage lipid-water (K_{SW}), membrane-lipid water (K_{MW}), protein-water (K_{PW}), and serum albumin-water partitioning (K_{BSA}) are significantly different than K_{OW} values and the associated proportionality constants for scaling octanol to these biological phases, e.g., (Henneberger 2016; Droge 2019; Allendorf 2021). Referring to chemical-specific biopartitioning information is encouraged to determine if the assumption of using octanol as a surrogate for biological partitioning is sufficient and if this assumption is not appropriate then biological partitioning data are required. Methods for measuring and predicting biological partitioning properties are available, e.g., (Endo 2013; Endo 2014; Ulrich 2017; Goss 2018; Droge 2019).

4.2 Physical-Chemical Properties – Ionizable Organic Chemical (IOC)

As with neutral organic chemicals, the same categories of physical-chemical property information are needed for IOCs. However, IOCs can exist in neutral and charged forms in the environment, as determined by the pH of the system (biological or environmental) and the dissociation constant(s) of the IOC (pKa). Therefore, the pKa and apparent partitioning information for the charged form are required to confidently assess IOC bioaccumulation. For many IOCs that are appreciably ionized at environmental and physiological pH, estimates of biological partitioning are preferred over octanol based partitioning information. While there has been significant scientific improvement for mass balance bioaccumulation models and biopartitioning data of IOCs over the past 10 years, e.g., (Ng 2013; Bittermann 2014; Ng 2014; Henneberger 2016; Armitage 2017; Goss 2018; Droge 2021; Arnot 2023; McLachlan 2023), there are very limited measurements and evaluated models for estimating biological partitioning for the charged form of an IOC and addressing this uncertainty requires integrated testing strategies for these properties and model evaluations. Bioaccumulation models that can explicitly consider the biopartitioning of the neutral and charged forms of IOCs like those in the BAT and BET are theoretically most appropriate for simulating bioaccumulation of IOCs in aquatic and air-breathing organisms.

4.3 Biotransformation and Elimination Rates

Biotransformation is a key process mitigating the bioaccumulation potential of organic chemicals in aquatic and terrestrial organisms (Armitage 2007; Arnot 2008a; Kim 2016; Walters 2016). For chemicals with high bioaccumulation potential the total elimination rate constant is often largely or entirely determined by the biotransformation rate constant. Biotransformation is thus a crucial consideration for refining the B assessment that would otherwise be based only on equilibrium partitioning data (e.g., K_{OW}) and is therefore a vital component of the overall B assessment. An indication of biotransformation potential is useful, but the process needs to be expressed quantitatively in terms of rate constants or half-lives for integration in B models or for comparisons with proposed thresholds for rate constants or half-lives. As outlined in the sub-sections below there are several LoE for biotransformation and terminal (total) elimination rate constants including i) empirical *in vivo* data, ii) *in silico* data (i.e., QSAR predictions), and iii) *in vitro* data (S9, hepatocyte, microsomal) and subsequent *in vitro-in vivo* extrapolation (IVIVE) using models.

4.3.1 Empirical In Vivo Biotransformation and Elimination Rate Constants and Half-Life Data

Databases of whole-body first order biotransformation rate constants (k_B) and corresponding half-lives (HL_B) for humans and fish estimated from *in vivo* studies have been developed (Arnot 2008b; Arnot 2008c; Arnot 2014). Whole-body biotransformation rate constants (k_B) can be determined from *in vivo* data using a mass balance TK modeling method (Arnot 2008c). Briefly, the method simulates passive chemical uptake and elimination rate constants (and their associated uncertainties) and calculates k_B (and its associated uncertainty) from *in vivo* bioaccumulation or clearance studies. The method was first applied to critically evaluated measured bioaccumulation and TK data for fish and a database is available (Arnot 2008b; Arnot 2008c). This database was subsequently used to develop several HL_B-QSARs. The *in vivo* k_B estimation method was subsequently adapted for estimating k_B (and HL_B) in mammals and applied to critically evaluated *in vivo* HL_T data for humans to develop a HL_B database for humans (Arnot 2014). These *in vivo* databases were then used to develop several HL_B- and HL_T-QSARs. The fish and human HL_B and HL_T data published by Arnot and colleagues are available at www.eas-e-suite.com and in the OECD QSAR Toolbox (https://www.oecd.org/en/data/tools/oecd-qsar-toolbox.html). Other methods for estimating *in vivo* biotransformation rate constants have been published and could also be considered, e.g., (de Wolf 1993; Gobas 2016; Lo 2016). Building from the human HL_T database of Arnot and colleagues (Arnot 2014), a mammalian TK database has also been developed by ECHA (Hofer 2021). Depending on the model being used to calculate B metrics, e.g., Equation 3, either k_B or k_T data can be used; however, it is important to recognize that k_T includes all elimination rate processes.

4.3.2 In Silico Whole-Body Biotransformation Rate Constants and Half-Life Data

Several validated *in silico* (QSAR) models for predicting HL_B for fish and predicting total elimination half-lives (HL_T) and HL_B for humans developed from *in vivo* data exist and are publicly available (Arnot 2009; US EPA 2011; Brown 2012; Papa 2014). The corresponding QSARs have been developed and evaluated following OECD QSAR guidance (OECD 2004a, 2007). There are a few approaches for predicting HL_B for organic chemicals in the literature. For example, the first HL_B-QSAR developed is in the BCFBAF v3.01 module of EPI Suite™ v4.11 (Arnot 2009). Other HL_B-QSARs for fish were developed and validated following OECD guidance for QSAR applications in regulatory decision-making (OECD 2007). These include the IFSQSARs developed by Brown et al. (Brown 2012) available in www.eas-e-suite.com, the US EPA OPERA QSARs implemented in the EPA Chemistry Dashboard, https://comptox.epa.gov/dashboard/, and several HL_B-QSARs developed at the University of Insubria (Papa 2014a), which are available as a downloadable software package (QSARINS, http://www.qsar.it/) and on-line www.eas-e-suite.com. The *in vivo* HL_T and HL_B databases for humans have been used to develop and validate HL_T and HL_B-QSARs for humans (Arnot 2014; Papa 2018), following OECD guidance for QSARs used in regulatory decision-making (OECD 2007). The HL_T- and HL_B-QSARs for humans are available on-line at www.eas-e-suite.com.

4.3.3 In Vitro Biotransformation Data – Liver S9, Hepatocyte, Microsomes (Liver)

Methods for measuring *in vitro* biotransformation rates in various tissues from various species and converting these rates to *in vivo* rates (liver clearance, whole-body) have been developed and applied for decades (Wilkinson 1975; Rane 1977). Standardized *in vitro* biotransformation rate methods for fish S9 (Johanning 2012) and hepatocyte (Fay 2015) assays have evolved. *In vitro* biotransformation studies using liver cells (primary cryopreserved hepatocytes) and liver subcellular fractions (S9 or microsomes) are becoming increasingly common in ecotoxicology (Johanning 2012; Arnot 2014; Fay 2017; Nichols 2018a).

Two OECD test guidelines to measure *in vitro* biotransformation in rainbow trout liver S9 subcellular fractions and cryopreserved hepatocytes are now available as is a publication documenting the results of an international ring trial for biotransformation rate estimation using cryopreserved hepatocytes and liver S9 (OECD 2018b, 2018c, 2018a).

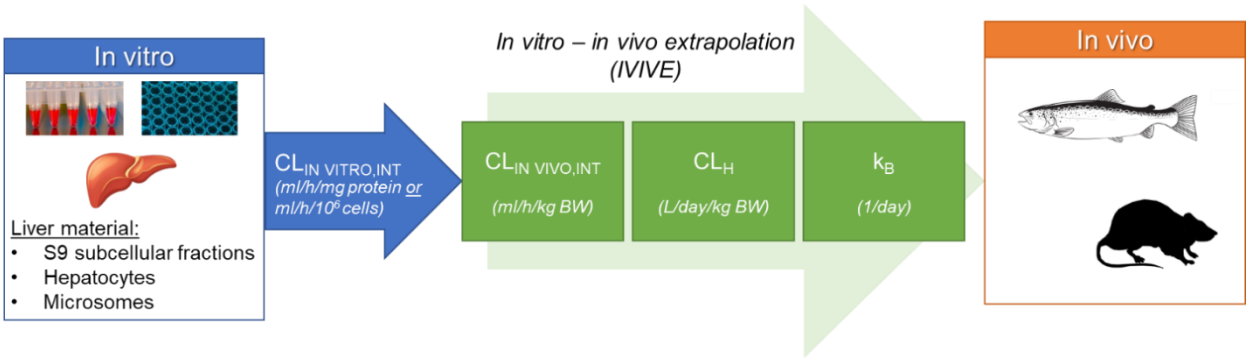
The main objective of these tests is to obtain an estimate of the first-order depletion rate constant of the parent chemical in the test system (k_e). This rate constant can be measured from the loss of the parent chemical (preferable) or inferred from the rate constant for product formation at test concentrations significantly below saturable (i.e., Michaelis-Menten) enzyme kinetics. The overall goal of *in vitro* biotransformation data generation is to estimate an intrinsic *in vitro* clearance rate ($CL_{IN\ VITRO,\ INT}$). As outlined in the next section, the *in vitro* first-order depletion rate constant can subsequently be converted using IVIVE models to assessment-relevant metrics such as whole-body biotransformation rate constant (k_B) and half-life (HL_B) as $HL_B = \ln 2/k_B$. This approach relies on the assumption that the liver is the main site of biotransformation in the body and (with IVIVE) can be used to approximate the whole-body k_B if there is no significant extrahepatic biotransformation.

QSARs developed for predicting clearance rates from *in vitro* data also exist, e.g., (Berellini 2012; Pirovano 2016), and some of these models are publicly available, e.g., the In Vitro Biotransformation Prediction (IVBP) Suite at the University of Insubria (https://dunant.dista.uninsubria.it/qsar/).

4.3.4 In Vitro - In Vivo Extrapolation

Methods for extrapolating the *in vitro* rates to *in vivo* rates have also evolved in human health, e.g., (Riley 2005; Barter 2007) and ecological sciences, e.g., (Han 2009; Laue 2014). **Figure 4** shows the general concepts for *in vitro - in vivo* extrapolation (IVIVE) of biotransformation rates. The main steps required are to i) convert intrinsic *in vitro* clearance ($CL_{IN\ VITRO,\ INT}$) to intrinsic *in vivo* clearance ($CL_{IN\ VIVO,\ INT}$), ii) convert intrinsic *in vivo* clearance ($CL_{IN\ VIVO,\ INT}$) to hepatic clearance (CL_H), and iii) convert hepatic clearance (CL_H) to a whole-body biotransformation rate constant (k_B). Hepatic clearance (CL_H) accounts for blood flow to the liver as the rate-limiting process and the conversion to k_B accounts for the distribution of the chemical in the body. The k_B estimate can then be used in models for calculating B metrics for fish like BCFs, e.g., (Han 2009; Laue 2014) and BMFs, e.g., (Saunders 2020b; Saunders 2023a) and BMFs for mammals, e.g., (Lee 2017).

Figure 4. Conceptual overview of the IVIVE calculation for estimating biotransformation rate constants (k_B) and subsequent whole-body biotransformation half-lives (HL_B) from in vitro bioassays (ref: www.eas-e-suite.com).



Depending on the IVIVE model there can be various parameters required for the calculations. Typically, these parameters include:

- The *in vitro* depletion rate constant k_e.
- Organism physiological parameters (liver weight as fraction of body weight, total cardiac output, fraction of cardiac output to liver) and compositions of the whole organism and blood (lipids, proteins, water). Default parameters can be used and are defined within specific models.
- Blood-water partitioning data: i) equilibrium partitioning approach (Lee 2017; Krause 2018; Saunders 2020a; Krause 2021) ii) regression-based approach (Fitzsimmons 2001) or iii) user-defined. The regression approach (Fitzsimmons 2001) was derived for neutral organic chemicals and is not recommended for IOCs. The equilibrium model or user-defined values should be used for IOCs.
- A value for the ratio of unbound fraction in blood and the test system (f_U) either as an explicit calculation (e.g., $f_U = f_{U,Bl} / f_{U,S9}$) (Nichols 2006; Nichols 2013; Krause 2018) or a user-defined value.

Reviews of data and models for estimating fraction unbound in *in vitro* and *in vivo* systems are available and systematic testing guidance for addressing the uncertainty in these parameters is available, e.g., (Krause 2021). While there are various IVIVE models available, the ones used in this IATA are coded and publicly available in the BET in EAS-E Suite (www.eas-e-suite.com) and the BAT (ARC Arnot Research and Consulting 2019; Arnot 2023). It is recognized that other IVIVE models have been developed or are under development, e.g., (Stadnicka-Michalak 2014; Krause 2018) and future IVIVE models may allow for incorporation of other approaches and parameterizations.

If multiple LoE are available for biotransformation rates (i.e., *in vitro*, *in silico*) the BAT calculates the geometric mean of reliable values, and the variability of these values is expressed as an uncertainty factor. This uncertainty factor is then used to propagate the uncertainty in biotransformation in the B metrics calculated by the models built into the BAT.

4.3.5 Biotransformation in Additional Species / Taxa

Biotransformation rate constants and half-life data and models have been predominantly developed for fish and mammals and are not available for all ecological species. These data gaps can be addressed by applying allometric scaling models and, if desirable, uncertainty factors for inter-species scaling. Toxicokinetic parameters like k_T and HL_T are a function of body size, e.g., (Hendriks 2001); therefore, allometric relationships are commonly used to convert data for applications in various models and regulatory decision-making. The general allometric scaling equation is:

$$k_x = k_Y \times (M_Y / M_X)^{0.25} \quad \text{(Equation 4)}$$

where k_x is the rate constant for the organism of interest (x), k_Y is the rate constant in another organism with a body mass M_Y (kg) and M_X is the body mass (kg) for the organism of interest. Following this general approach, biotransformation rate constant data can be normalized to a specific body size and temperature (e.g., 0.01 kg for a fish) and this is referred to as $k_{B,N}$ (and the associated body and temperature normalized half-life, $HL_{B,N}$). For inter-species extrapolation and modeling purposes, the HL_B for a particular organism (x) with body mass M_X can be scaled for differences in body mass as:

$$HL_B = HL_{B,N} \times (M_N / M_X)^{-0.25} \quad \text{(Equation 5)}$$

Note the exponent in this allometric relationship is negative in this case using half-lives. There is uncertainty in allometric scaling; however, this method is consistent with common practice in the pharmaceutical and veterinary sciences and when extrapolating rodent data to human data for health assessment. The commonly assumed allometric scaling relationship is suggested in this IATA unless chemical- and biota-specific values are available.

Applying uncertainty factors for species differences in TK is common practice in human health assessment, e.g., (US EPA 2014). For example, when using this IATA, a scaling (uncertainty or assessment) factor of 3 or 10 could be applied when extrapolating HL_B from fish to aquatic invertebrates. This scaling factor method implies that "all else being equal" the biotransformation half-life for a chemical in an invertebrate is 3 (or 10) times longer (slower) than in a fish of the same mass and system temperature. A similar, or user-defined, scaling factor could be applied when scaling toxicokinetics data from mammals to birds. Improving knowledge for inter-species differences in biotransformation rates is considered a priority research need to address uncertainty in TK across species. Default inter-taxa scaling factors are included in the BET and the BAT for converting HL_B from fish to invertebrates and mammals to birds and users can refine these scaling factors as desired.

4.4 Bioaccumulation Data

This section refers to various B assessment metrics that can be obtained from multiple sources (*in vivo*, *in silico*; laboratory, field) and can be used directly within the IATA and included as part of the WoE.

4.4.1 In Silico Bioaccumulation Models

In silico methods for predicting the B metrics commonly used in B assessment and used in this IATA are listed first because these data sources are more readily available for a chemical subject to B assessment. Details of these different B metrics are provided in the sections below relating to their measured derivation following OECD standard test methods. All modeling applications and results used in the IATA should follow the guidance principles of good modeling practice as outlined by Buser et al. (Buser 2012).

The fish BCF has traditionally been the most commonly and widely used endpoint for B assessment and there have been several BCF models developed over the past few decades. In addition to QSAR models for fish BCFs there are mass balance models for simulating BCFs, BAFs, BMFs, HL_T and TMFs in a range of ecological receptors and food webs. This IATA cannot list all BCF, BAF, and BMF models that are currently available; however, some sources for finding these models include:

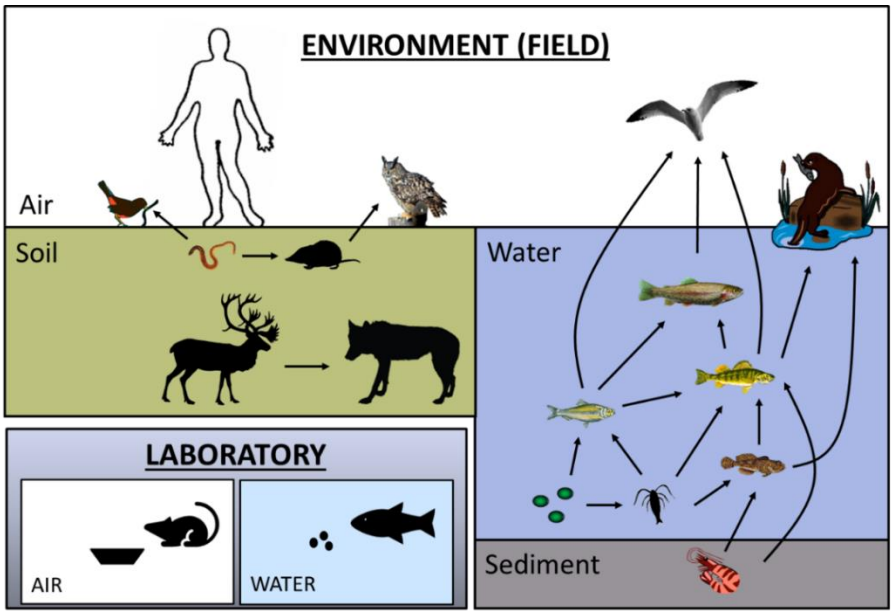
- BCF Base-Line Model: http://oasis-lmc.org/products/models/environmental-fate-and-ecotoxicity/bcf-base-line-model-(1).aspx
- Bioaccumulation Evaluation Tool (BET): https://www.eas-e-suite.com
- ADME-B(ioaccumulation) Calculator: A Toxicokinetic Framework and Analysis Tool for Interpreting Organisation for Economic Co-operation and Development Guideline 305 Dietary Bioaccumulation Tests (Gobas 2019b)
- EPI Suite BCFBAF: https://www.epa.gov/tsca-screening-tools/epi-suitetm-estimation-program-interface
- KABAM: https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/kabam-version-10-users-guide-and-technical

- OECD QSAR Toolbox: https://www.oecd.org/en/data/tools/oecd-qsar-toolbox.html
- OPERA: https://github.com/NIEHS/OPERA
- TEST: https://www.epa.gov/chemical-research/toxicity-estimation-software-tool-test
- VEGA CAESAR: https://www.vegahub.eu/portfolio-item/vega-qsar/

One can choose BCF, BAF and BMF models of their preference; however, the models should be evaluated for their reliability and suitability as outlined below in **Section 5**.

In this IATA we focus on the BET module in EAS-E Suite because it is publicly available, includes a range of organisms and B metrics and is readily parameterized in the EAS-E Suite platform. **Figure 5** provides a conceptual overview for most of the representative species in the BET and the built-in modeling component of the BAT. The BET includes representative aquatic and terrestrial organisms in laboratory settings and in environmental food webs and one only needs a chemical CAS, name, or SMILES notation to obtain BCFs, BAFs, BMFs and HL_T in representative ecological species. The models provide calculated BCFs and BAFs based on the total and dissolved water concentrations and calculated BMFs based on wet-weight, lipid-weight or chemical activity ratios (Arnot 2022). The models in the BET are consistent with the models built into the BAT for comprehensive WoE B assessments when multiple LoE from various sources are available and there is an unacceptable lack of consistency across the LoE B assessment outcomes for the evaluator to make a decision with sufficient confidence. Thus, one can proceed from using the screening-level BET to the more data-intensive BAT with the same concepts, models, and B metrics, and with additional measured and predicted B data. Of course, one can also obtain model predictions for B metrics other than those provided by the BET, i.e., fish BCFs, as outlined in the examples (**Section 7**) used to demonstrate this IATA.

Figure 5. Conceptual overview of the representative aquatic and terrestrial organisms included in the Bioaccumulation Estimation Tool (BET) model in EAS-E Suite (www.eas-e-suite.com) and the bioaccumulation models built into the Bioaccumulation Assessment Tool (BAT) (Arnot 2023).



The BET can be parameterized in EAS-E Suite with user supplied CAS, chemical name, or SMILES notation. The default BET model input parameters initially provided by EAS-E Suite (e.g., partition ratios, biotransformation half-lives) can readily be replaced with user-preferred values. The BAT can be used in a WoE approach when additional LoE are available, e.g., measured lab and field B metrics.

4.4.2 Laboratory BCF Data

The OECD 305 Testing Guidelines for aquatic bioaccumulation testing in fish (OECD 2012) are well-established and recognized by various regulatory authorities. The original OECD 305 Testing Guidelines were for determining the BCF in fish. In the OECD TG 305 aqueous test, fish are only exposed to the chemical in the water. The BCF is calculated at steady-state as the ratio of the chemical concentration in the organism (C_B) and the aqueous phase (C_W), i.e., $BCF_{SS} = C_B / C_W$. For the BCF_{SS} to be valid, the concentration of the chemical in the aqueous phase must be maintained throughout the uptake period and the concentration of the chemical in the fish must approach steady-state. The kinetically-derived BCF is calculated as the ratio of the gill uptake rate constant (k_1, L/kg/d) and the total elimination rate constant (k_T, 1/d), i.e., $BCF_K = k_1 / k_T$. For the BCF_K to be valid the concentration of the chemical in the aqueous phase must be maintained throughout the uptake period and elimination of the chemical during the depuration phase must follow first-order kinetics (OECD 2012).

If desirable, additional BCF metrics can be calculated from the experiments, if growth rates or lipid contents are reported. For example, the lipid-standardized steady-state BCF ($BCF_{SS,L}$), growth-corrected kinetic BCF ($BCF_{K,G}$), lipid-standardized kinetic BCF ($BCF_{K,L}$), growth-corrected and lipid-standardized kinetic BCF ($BCF_{K,L,G}$) metrics can be calculated. In this context the lipid-standardized BCFs are calculated by dividing the whole-body wet weight BCF by the whole-body lipid content of the fish on a mass fraction basis, see the OECD 305 Testing Guidelines (OECD 2012) for more details. The credibility of growth-correction for the BCF has been questioned and is generally not recommended (Gobas 2019a).

The BCF can be expressed on the total water concentration in the exposure system or the freely dissolved water concentration. Only the freely dissolved fraction of the total chemical in the water is bioavailable for uptake into the organism (Barron 1990). The dissolved fraction is a function of the water properties and the chemical properties, e.g., (Parkerton 2008). As organic matter and chemical hydrophobicity increase, the freely dissolved fraction decreases. Therefore, this can be a confounding factor when comparing BCFs across a range of exposure conditions and chemicals. Thus, there is interest in determining the BCF on the freely dissolved concentration. For very hydrophobic chemicals (i.e., log K_{OW} > 6), determining the true freely dissolved concentration in the bulk water sample can be technically challenging. This technical difficulty was one of the motivating factors for revising the OECD 305 Testing Guidelines to include dietary exposures and BMF testing. In fact, for such hydrophobic chemicals, dietary exposures are recommended over aqueous exposures (OECD 2012).

In Europe, bioaccumulation data for invertebrates are used in regulatory decision-making, including PBT assessment. A *Hyalella azteca* Bioconcentration Test (HYBIT) has been adopted by OECD to provide a non-vertebrate test for bioconcentration in aquatic environments (OECD, 2024).

4.4.3 Laboratory BMF Data (Aquatic)

The OECD 305 Test Guidelines for aquatic bioaccumulation testing are well-established and recognized by various regulatory authorities (OECD 2012). While the 305 TG were originally developed for determining the BCF in fish, in 2012 the guidelines were modified for determining the BMF from dietary exposures. In the OECD TG 305 dietary test, fish are only exposed to the chemical in the diet. Directly applicable B

metrics that can be calculated from the test are the BMF_{SS}, BMF_K, and $BMF_{K,L}$. If growth rate data are available from the experiment, growth-corrected BMFs can also be derived (e.g., BMF_g), if desirable; however, the credibility of growth-correction for the BMF has been questioned and is generally not recommended (Gobas 2019a). Other relevant TK parameters in fish, i.e., dietary absorption efficiency, and k_T, can also be quantified in the 305 *in vivo* test. Laboratory BMFs are not the same as field BMFs because field BMFs include co-exposure to the chemical in the water. This confounding issue can be addressed in the BET and the BAT because both lab and field BMF measurements and model predictions can be considered.

4.4.4 Laboratory Toxicokinetic (TK) and BMF Data (Mammals)

Dietary uptake testing in mammals like laboratory BMF tests with fish for determining bioaccumulation metrics are rare; however, there are studies reporting TK data that are useful for B assessment. These include the total elimination rate constant (k_T) or half-life (HL_T) derived from mammalian TK studies (e.g., OECD TG 417). While standard testing for BMFs in mammals does not exist, the same principles of the fish BMF testing guidelines can be applied to calculate the BMF with the following (kinetic) expression:

$$BMF_K = \frac{I \cdot E_D}{k_T} = \frac{k_D}{k_T} \quad \text{(Equation 6)}$$

where I is the feeding rate normalized to body size (g-food/g-organism/d) and E_D is the chemical uptake efficiency from the gastrointestinal tract (GIT). Note that k_T can be estimated from half-life as $\ln 2/HL_T$. Like the fish BMF, the mammalian BMF should be lipid-normalized or expressed on a chemical fugacity or chemical activity basis to determine the thermodynamic status of the chemical between the organism and its food. For IOCs, the chemical activity ratio is generally considered more appropriate for BMFs (Arnot 2022).

4.4.5 Field BAF Data

Field data are often considered the gold standard for chemical assessments; however, they are resource-intensive to obtain and are not readily available for many chemicals requiring evaluation. One historical B metric for aquatic organisms is the BAF. The BAF is the steady-state ratio of the chemical in the organism compared to the surrounding water, i.e., $BAF_{SS} = C_B / C_W$, in the natural environment. It can be calculated from the total (BAF; L-water/kg-ww) or freely dissolved (BAF_{fd}; L-dissolved/kg-ww) chemical concentration in the water and from the whole-body weight or the lipid-normalized body weight, e.g., $BAF_{L,fd}$; L-dissolved/kg-lw. Steady-state conditions are often assumed, but efforts to confirm this requirement for a reliable calculation are suggested. There are several other challenges to obtaining relevant and reliable field B metrics like the BAF, e.g., sufficient samples sizes with quantifiable chemical concentrations, sufficient temporal and spatial considerations. There is limited detail in existing guidance documents (ECHA 2016, 2017a) about how such information should be interpreted, along with any technical considerations (e.g., preferred methods) and pitfalls to consider. Some preliminary guidance for the reliability of field data is provided in **Section 5** below.

4.4.6 Field BMF Data

A field BMF can be calculated from biomonitoring data as the steady-state ratio of the chemical concentration in the organism compared to its diet, i.e., $BMF_{SS} = C_B / C_D$. It can be calculated from wet weight concentrations but is preferably calculated based on lipid-normalized concentrations or fugacity (activity) ratios, e.g., BMF_L (Gobas 2009; Burkhard 2012; Arnot 2022). For more hydrophobic chemicals with sufficient quantification confidence in samples, the field BMF can often be determined with greater confidence than the BAF because of the uncertainty in determining freely dissolved water concentrations; however, many of the sources of uncertainty in field BAFs also exist for field BMFs.

4.4.7 Field TMF Data

While field BMFs are dependent on the specific trophic interaction under study (i.e., a defined predator/prey relationships), the TMF is effectively an average of the BMF across one trophic level over the course of the studied food web. It is thus a function of the specific food web and the environmental conditions, e.g., sediment-water disequilibrium, and variability in TMFs between ecosystems is expected and observed, e.g., (McLeod 2015; Franklin 2016; Kim 2016; Mackay 2016; Fremlin 2021). The TMF is also the most resource-intensive B metric to empirically quantify.

5 Data Evaluation & Weighing

The next phase of the B IATA includes both data evaluation and data weighing. The first of these two steps provides a transparent approach, using Data Evaluation Templates (DETs) to critically evaluate each LoE for reliability and uncertainty. The second, data weighing phase, allows a "weight" to be assigned to each LoE by combining the relevance score that was assigned *a priori* within the problem formulation phase with the results from the data evaluation phase. This approach generally follows OECD WoE guidance (OECD 2019).

5.1 Data Evaluation

All data that can be used in this IATA, whether measured or predicted, are inherently uncertain. Some sources of uncertainty include deviation from standardized protocols (contributing to variability and/or error), insufficient reporting of supporting data to understand how key parameters influence the results or marginalize the reproducibility of the results, experimental or technical errors, or limited statistical significance. The overarching objective for developing and applying data quality assessment methods is to identify uncertainty and guide the selection and application of the best available information providing confidence in the decision. Data reliability assessment methods cannot guarantee that all uncertainty in the data have been identified - uncertainty in the data will remain; however, relevant data quality issues can be identified, considered, and transparently communicated to stakeholders to support the decision. Various methods and criteria have been developed for evaluating ecotoxicological and bioaccumulation data, e.g., (Klimisch 1997; Arnot 2006; Parkerton 2008; Arnot 2015). In this IATA we refer to DETs that have been prepared in recent years through consultation with stakeholders in the development of the guidance and methods for a WoE approach to bioaccumulation assessment (Arnot 2023). The DETs can be used for screening-level assessment applications of this IATA (e.g., for evaluating the reliability of a QSAR prediction) or in a comprehensive WoE approach for definitive B assessment with multiple LoE. When using this IATA, one is not strictly mandated to follow each criterion of each DET, they are provided as general, yet recommended, guidance based on OECD Testing Guidelines for specific and relevant data types (i.e., LoE) and expert opinion. Like all methods and criteria, the methods and criteria that form the DETs are likely to evolve with the science and application experience. The development of OECD Testing Guidelines has been more involved for laboratory data endpoints (i.e., BCF, BMF) compared to some other LoE (i.e., field data and QSARs). To the greatest extent possible these DETs were developed from standardized OECD TGs and applying expert judgement as necessary (e.g., when B metrics do not have OECD guidance). Most of the DETs are also integrated into the BAT to facilitate the operation of the guidance provided (ARC Arnot Research and Consulting 2019; Arnot 2023). The following section briefly summarizes the rationale for each DET and the criteria in the BAT Ver.2.02 and its accompanying user manual. The DETs below do not include quantitative values for each of the data quality criteria therein; however, the DETs incorporated in the BAT include quantitative scores for each criterion. The DETs in the BAT also

include criteria and conditions that result in a “critical fail” of the LoE and in such instances the data should not be used for decision-making.

5.1.1 Data Evaluation of QSAR Predictions

Table 4 is a DET considered representative for *in silico* QSAR model predictions and is based on OECD guidance principles for using QSARs in regulatory decision-making (OECD 2004b, 2004a, 2007). This general QSAR DET can be considered for various QSAR predicted endpoints including, but not limited to, K_{OW}, K_{OA}, BCF, HL_B and HL_T. More recent guidance on QSAR model application is available in OECD QSAR Assessment Framework (QAF) which provides guidance on the assessment of QSAR models as well as their predictions (OECD 2023b). The QAF guidance was published after the drafting of this IATA; however, the general principles in the QSAR DETs are well-aligned with the recent OECD QAF.

Table 4. Data quality considerations for in silico QSAR model predictions in the form of a Data Evaluation Template (DET)

Quality Criterion/Consideration
Is a defined endpoint clearly presented?
Is the (Q)SAR expressed in the form of a transparent and unambiguous algorithm?
Is the (Q)SAR associated with appropriate measures of goodness-of–fit, robustness and predictivity?
Is the (Q)SAR associated with a defined domain of applicability?
Does the (Q)SAR provide a mechanistic interpretation for the estimate?
Is the prediction within the stated applicability domain of the QSAR?
Was the (internal validation) $r^2 > 0.7$?
Was the (external validation) $q^2_{ext} > 0.5$?
Critical Fail for other reason (override; quality score = 0). The user should provide a brief statement in the space provided justifying this decision.

Source: ARC Arnot Research and Consulting 2019; Arnot 2023.

5.1.2 Data Evaluation of In Vitro Biotransformation Experiments

Table 5 is a DET developed to evaluate the reliability of fish or mammalian *in vitro* biotransformation data. **Table 5** is a representative DET for *in vitro* biotransformation data (liver S9), while additional DETs have been developed for other assay types (hepatocytes, microsomes) and are available (ARC Arnot Research and Consulting 2019; Arnot 2023).

Table 5. Data quality considerations for fish or mammalian in vitro biotransformation data in the form of a Data Evaluation Template (DET)

Quality Criterion/Consideration
Consideration for LOQ: measured concentrations are > LOQ or C_0 > 10LOQ, < LOQ or not reported.
Enough independent experiments/runs? >=2 or <2 independent experiments, >=2, <2 replicates or unknown?
Statistical quality is high (r^2>0.85 and significant slope, number of timepoints > 6), low (not significant) or not reported (but assumed OK)
What was the chemical purity?
Was the biological material characterized, and with high confidence, e.g., activity of EROD, UGT, etc?
Was the assay duration appropriate?
What was the concentration of vehicle (spiking solvent) used? Was it DMSO > 0.5% (CRITICAL FAIL)
Was a positive control or a reference chemical used?
Were non-metabolic losses predicted to be significant based on estimates using an in vitro mass balance model (IV-MBM)? No (loss <= 20%, possible (loss > 20% and <=67% or not characterized/run/reported?
Was a negative control used and what were the (loss) results?
Was the initial test concentration (C_0) reported?
Was the cell concentration (C_{cell}) reported?
Was initial concentration (C_0) < Michaelis-Menten constant (k_{MM})? OR were first order kinetics confirmed?
Was the estimated assay medium concentration (C_{free}) based on IV-MBM in comparison to the chemical water solubility limit (S_W)?
What was the rate determination method? Substrate depletion (SD), confirmed or assumed or product formation (PF), confirmed or assumed.
Were the assay conditions consistent with *in vivo* (pH, temp, co-factors added)?
Was $CL_{InVitro,Int}$ reported or readily calculated with reported data (i.e. $C_{cell}/C_{protein}$)?
Were the units presented clearly with unit conversions needed or were assumptions about the units made?
What was the statistical difference from the control?
Critical Fail for other reason (override; quality score = 0)

Source: ARC Arnot Research and Consulting 2019; Arnot 2023.

5.1.3 Data Evaluation of In Vivo BCF Experiments

Table 6 is a DET developed for evaluating experimental (laboratory) BCF data that is based on requirements outlined in the OECD Technical Guidance Document for fish (OECD 2012) and other recognized key sources of uncertainty determined from evaluating thousands of experimental BCFs for invertebrates, algae and fish (Arnot 2006; Parkerton 2008). The template was developed to provide a general assessment of data reliability, not solely to evaluate whether a test satisfies all OECD 305 test requirements. Most of the criteria below are suitable for evaluating the data quality of BCF experiments using invertebrate species.

Table 6. Data quality considerations for experimental BCF data in the form of a Data Evaluation Template (DET)

Quality Criterion/Consideration
BCF units clearly reported
BCF for parent chemical reported
If BCF was calculated as C_{Fish}/C_{Water}, was the steady-state assumption ("within 20%") confirmed? (otherwise N/A)
If BCF was calculated as k_1/k_T, were the rate constants with units clearly reported? (otherwise N/A)
Fish concentration measured directly for chemical of interest?
For ionisables, was pH reported and within 0.5 log units of average? (otherwise N/A)
Estimated dissolved water concentration (C_{Free}) with respect to Water Solubility (S_W)
Water Concentration measured directly for chemical of interest?
Water Concentration within ± 20% of nominal throughout exposure?
For log K_{OW} > 6, was total organic carbon (TOC) reported and less than 2mg/L? (otherwise N/A)
Mortality/adverse effects in test/control group < 5%
Whole-body fish lipid content reported?
Test species reported?
Fish mass reported?
Whole-body fish analyzed?
For chemicals with log K_{OW} > 6, was growth rate reported?
Was there a control group?
What was the chemical purity?
LOQ reported?
Study conducted according to recognized international standard e.g., OECD305?
Study consistent with GLP or similar guiding principles?
Test design (flow through, semi-static, static, not reported)
Water temperature reported AND appropriate for species AND relatively constant (±2°C)
Test concentration < 1% reported acute toxicity?
For neutrals: was pH reported?
For log K_{OW} ≤ 6, was TOC reported and less than 2mg/L? (otherwise N/A)
Was dissolved oxygen reported and > 60% saturation?
Similar weight or length of fish used throughout study?
Acclimatization for at least 14 days under test conditions?
Feeding rate reported in the range of 1-3% body weight per day?
Minimum of 4 fish/sampling event?
Water hardness is reported AND 10-250 mg/L?
Light-dark cycle reported AND 12-16 h illumination?
Critical Fail for other reason (override; quality score = 0)

Source: ARC Arnot Research and Consulting 2019; Arnot 2023.

5.1.4 Data Evaluation of In Vivo BMF Experiments

Table 7 is a DET developed for evaluating experimental (laboratory) BMF data that is largely based on the requirement outlined in the OECD Technical Guidance Document for fish (OECD 2012) and a critical review

of hundreds of measured BMF data in fish (Arnot 2015). The template was developed to provide a general assessment of data reliability, not solely to evaluate whether a test satisfies all OECD 305 test requirements. Most of the criteria below are suitable for evaluating the data quality of BMF experiments using invertebrate species.

Table 7. Data quality considerations for experimental BMF data in the form of a Data Evaluation Template (DET)

Quality Criterion/Consideration
BMF units clearly reported
BMF for parent chemical reported
If BMF was calculated as C_{Fish}/C_{Diet}, was the steady-state assumption ("within 20%) confirmed? (otherwise N/A)
If BMF was calculated as $(I \cdot E_D)/k_T$, were the rate constants with units clearly reported? (otherwise N/A)
Fish concentration measured directly for chemical of interest?
Dietary uptake efficiency (ED or alpha) ≤100??
For ionisables: was pH reported and within 0.5 log unts of average?
Diet concentration measured directly for chemical of interest?
Diet lipid content reported?
Was growth rate reported?
Mortality/adverse effects in test/control group < 5%?
Whole-body fish lipid content reported?
Test species reported? Is it an OECD recommended species?
Fish mass reported? Yes, Partial (start and/or end) or No
Whole-body of fish analyzed?
Feeding rate reported and in the range of 1-3% body weight per day?
Was there a control group?
What was the chemical purity?
LOQ reported?
Study conducted according to recognized international standard e.g., OECD305?
Study consistent with GLP or similar guiding principles?
Test design (Flow through, semi-static, static, not reported)
Water temperature reported AND appropriate for species AND relatively constant (±2°C)
Diet concentration < 1% reported acute toxicity?
For neutrals: was pH reported and within 0.5 log units of average?
Was dissolved oxygen reported and > 60% saturation?
Similar weight or length of fish used throughout study?
Acclimatization for at least 14 days under test conditions?
Minimum of 4 fish/sampling event?
Water hardness is reported AND 10-250 mg/L? Light-dark cycle reported AND 12-16 h illumination?
Light-dark cycle reported AND 12-16 h illumination (or otherwise appropriate)?
Critical Fail for other reason (override; quality score = 0)

Source: ARC Arnot Research and Consulting 2019; Arnot 2023.

5.1.5 Data Evaluation of In Vivo Mammalian Toxicokinetic Experiments

To evaluate the quality of *in vivo* toxicokinetics data, i.e., total (terminal) elimination rate constants or half-lives, to include in a BMF calculation for a representative lab mammal, e.g., Equation 6, a DET has been developed (available in (ARC Arnot Research and Consulting 2019; Arnot 2023)) that is largely based on some of the requirements outlined in the OECD 417 Technical Guidance Document (OECD 2010b).

5.2.6 Data Evaluation of Field Measurements

The following text details the considerations that have been identified when performing evaluations of biomonitoring data under UK REACH (UK Government 2021). When reviewing biomonitoring data, the data that need to be considered include the following:

- Has the organism been clearly described in terms of sex, age, and overall condition?
- Have a representative number of organisms been sampled?
- What is the organism's habitat and food sources? Is the organism transient, e.g., migratory?
- Are locations of sampling clearly described?
- Are analytical methods for sample preparation and detection suitable? e.g. Have extraction efficiencies and detection limits been assessed and reported?
- Have stable isotope concentrations ($\delta^{15}N$ and $\delta^{13}C$) been reported. These can be used to describe both the trophic position of organisms and similarity of food sources.
- How are the measurements being presented? e.g., wet / dry weight, lipid normalized etc.

These points and others were considered in developing DETs for field BAF and BMF data as outlined in Arnot et al., (Arnot 2023). **Table 8** outlines a DET for evaluating the reliability of measured field BAF and BMF metrics based on recognized sources of uncertainty and expert opinion**. Table 9** presents the DET for TMFs and considers similar aspects for field measurements as well as expert opinion on TMFs (Borgå 2012; Conder 2012; Burkhard 2013). A key consideration for the application of TMFs in bioaccumulation assessment decision-making, but not included in the current DET, is whether the calculated TMF is statistically different from 1.

Table 8. Data quality considerations for field BAF and BMF data in the form of a Data Evaluation Template (DET)

Quality Criterion/Consideration
Field blanks used in the sampling?
Randomized sampling method employed?
Water (for BAF) and dietary (for BMF) samples used in B metric co-located and considered representative of the exposures?
Biological and environmental (e.g., water) samples used in B metric obtained in same year and season?
Confidence that steady-state is approximated (e.g., +/-~20%) on a score of 0-30 (30 being analytical confirmation of this assumption):
For neutral chemical BMFs: lipid contents in predator and prey are reported?
Analytical standards used in the analysis?
For the numerator (organism) concentrations: What was the frequency of detects (80-100%, 50-80% or <50%/unknown) and sample size (>=20, 5-20 or <=5/unknown)?
For the denominator (water or diet) concentrations: What was the frequency of detects (80-100%, 50-80% or <50%/unknown) and sample size (>=20, 5-20 or <=5/unknown)?
How are the measurements below the method detection limit (MDL) addressed? Statistically, by replacement or unknown?
Are sampled species names reported?
For each sampled species (i.e., fish or within a taxa / TL for lower TLs): are organism masses (or length or age) reported and similar (i.e., min. differences)?
Is environmental temperature reported?
For ionizables: was pH reported?
Critical Fail for other reason (override; quality score = 0)

Source: ARC Arnot Research and Consulting 2019; Arnot 2023.

Table 9. Data quality considerations for field TMF data in the form of a Data Evaluation Template (DET)

Quality Criterion/Consideration
Field blanks used in the sampling?
Randomized sampling design employed?
Biological samples used in B metric co-located and relevant for dietary relationships?
Biological samples used in B metric obtained in the same year and season?
Confidence that steady-state is approximated (~±20%)
Study includes sampling from a minimum trophic level range of 2.0 (i.e., TL 2.0-4.0)
Method used to derive trophic level provided, e.g., δ15 N /δ13 C stable isotope ratio data available and appropriate baseline organism used.
Method used to determine the TMF provided?
Study design incorporates reasonable balance with respect to sample numbers of lower- versus higher-trophic-level organisms?
Lower trophic level organisms included in sampling (e.g., non-vertebrates)?
Whole-body analysed? If tissue only analysed, was a correction (normalization) performed?
Sample concentrations normalized appropriately?
Analytical standards used in the analysis?
For the organism concentrations: What was the frequency of detects (80-100%, 50-80% or <50%/unknown) and sample size (>=20, 5-20 or <=5/unknown)?
How are measurements below the method detection limit (MDL) addressed? Using statistical or replacement methods, or unknown?
Sampled species names reported?
For each sampled species (i.e., fish or within a taxa / TL for lower TLs): organism mass (or length or age) reported and similar (i.e., min. differences)?
Environmental temperature reported?
For ionizables: was pH reported?
Critical Fail for other reason (override; quality score = 0)

Source: ARC Arnot Research and Consulting 2019; Arnot 2023.

5.2 Weighing Lines of Evidence (LoE)

At this stage of the IATA, outcomes of the relevance and reliability evaluations are summarized and used to assign a qualitative weight to each LoE used to address the defined problem statement(s). Again, a categorical approach (e.g., low, medium, high) is suggested, noting that this is a judgment-based (normative) process. Further guidance on assigning LoE weight with an example approach is provided in OECD 2019. The goal of this step is to transparently communicate how weight was assigned to various LoE to address the problem statements. The IATA application case examples provided in **Section 7** have examples of tables that can be used to communicate these principles **Tables 16, 17, 26, 27, 41, and 42.**

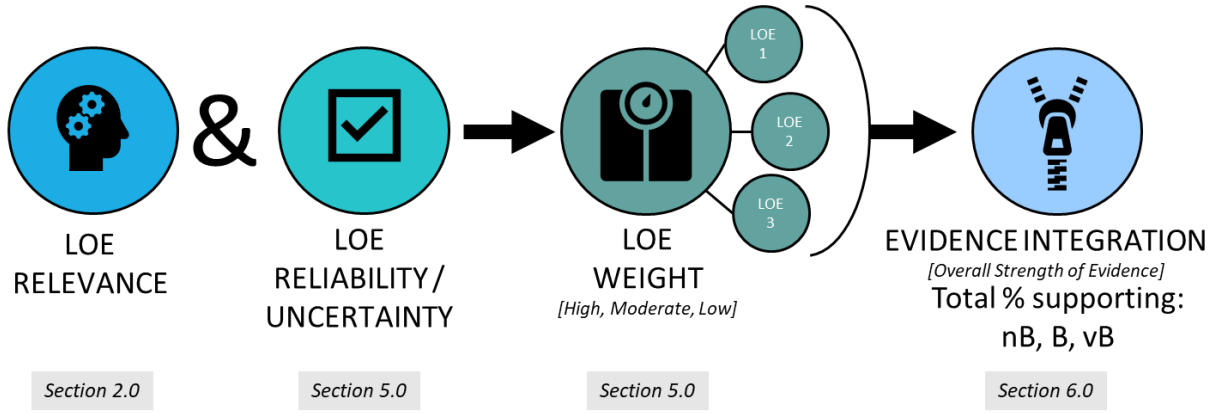
The number of weight categories is also subjective and can be selected according to the context of the problem (e.g., degree of categorical or numerical resolution, such as 3 or 5 or 10 categories). It should be noted that the user may wish to report all LoE including those that may not be directly relevant to the hypothesis so that reviewers can transparently see that they were considered.

Determining a categorical or numerical weight outcome in this step is situational. The assigned weight is a function of equal consideration given to relevance and reliability, such that given weights reflect a combination of these criteria. While guidance and examples are provided in this IATA and OECD guidance (OECD 2019) to judge the level of reliability and relevance for weighing bioaccumulation evidence, it is beyond the scope of this IATA to provide absolute rules for how these two WoE metrics should be used to assign weight. Such approaches will also be context dependent and could be developed by individual agencies for bioaccumulation.

6 Bioaccumulation Data Integration and Reporting

The last step of the B IATA is focused on evidence integration and reporting, consisting of three main parts: (1) Determination of overall strength of evidence (e.g., coherence) across all LoE; (2) assessment of the impacts of residual uncertainty; and (3) reporting the results. **Figure 6** provides a diagrammatic overview of the phases of the B IATA, showing how the earlier phases of LoE relevance and LoE data evaluation are combined to assign a "weight" to each LoE, which are then combined within this last phase of data integration and reporting.

Figure 6. Diagrammatic overview of the B IATA with focus on how the various LoE steps come together in the final, evidence integration stage.



6.1 Strength of Evidence (SoE)

This step of the IATA is designed to evaluate the overall strength of evidence (SoE), or consistency, across all the data elements (LoE). This process provides a means to evaluate the extent of variability among the LoE in terms of B assessment outcomes and identify possible outliers within a particular dataset. When data are more consistent, confidence is increased and can allow for values of central tendency or a selected percentile be used to represent multiple results (e.g., multiple bioaccumulation model predictions). The overall SoE is determined by the frequency of "B" categorization outcomes based on all LoE and is

expressed as a percentage. For example, if all LoE result in a “not B” categorization, the SoE for the chemical being “not B” is 100% and the SoE for the chemical being categorized as “B” or “vB” is 0%.

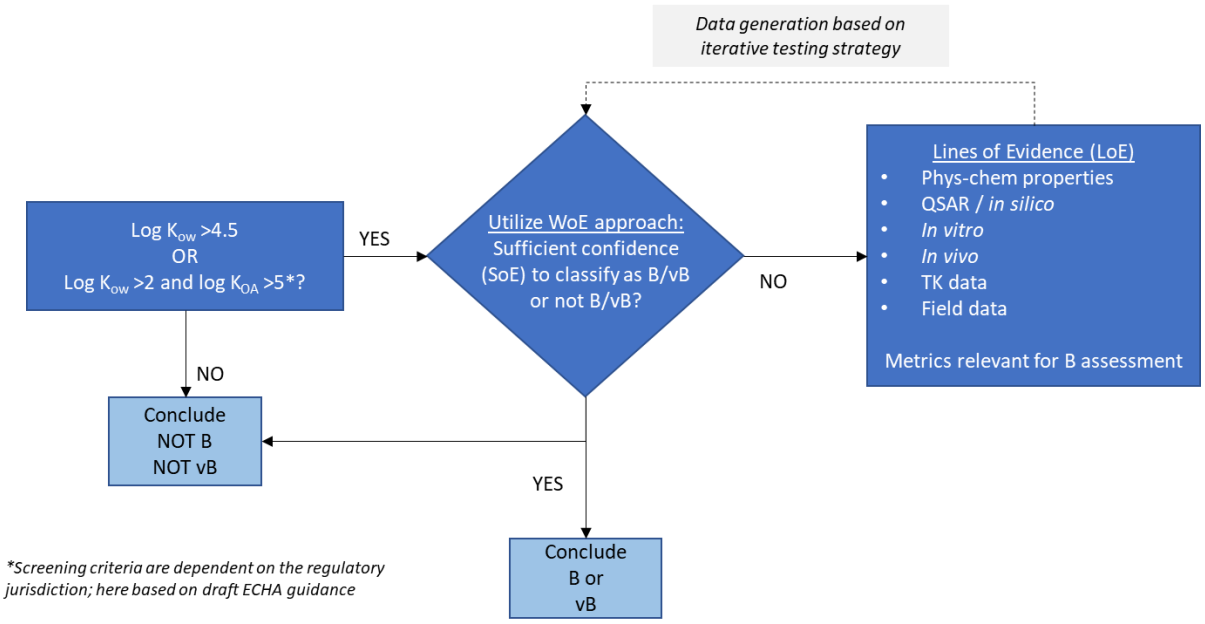
6.2 Evaluating Residual Uncertainty

The goal of this step is to determine if the available information as integrated into the SoE provides sufficient confidence to make a decision relating to the problem statement. **Figure 7** illustrates the decision-making process and iterative nature of this IATA. The diamond in the centre of the figure relates to this section of the IATA. The consistency within and among data sets can be examined to help arrive at this determination. True uncertainty generated via data gaps can trigger a reiteration step in the data generation phase of this IATA.

This stage of the IATA allows for identification of data and information gaps to assess data sufficiency and remaining (residual) uncertainty in the overall SoE. For example, it may be concluded at this stage that insufficient evidence is available to conduct the IATA and reach a conclusion to address the problem statement. If it is determined that there are insufficient data or too much uncertainty in the available data at this point, the evaluator can recommend further data generation. For example, if there are discrepancies in B categorization results (SoE < 100%) between various *in silico* BCF model predictions, a next pragmatic step would be to conduct an OECD *in vitro* biotransformation assay and subsequent IVIVE BCF calculations. This could be done for lower K_{OW} chemicals (log K_{OW} < ~6.5) that are within the AD of the *in vitro* bioassays. If the chemical hydrophobicity is greater, it may be more suitable to conduct an *in vivo* 305 dietary study. This step essentially determines if there is enough confidence in the gathered, evaluated and integrated data to make a B assessment decision, and if not, exploit the iterative framework of the IATA (**Figure 7**) to systematically obtain new data in the most efficient manner possible to address the remaining (residual) uncertainty in the data and the decision.

The assessment of uncertainty within each LoE is essential and is not considered a separate standalone exercise conducted once the IATA is complete. The IATA user is guided towards considering the uncertainties identified under the evaluation of data quality, sufficiency and consistency in relation to the problem statement(s). For example, if the problem statement is “Is there sufficient evidence to conclude that substance X has a BMF > 1 and therefore can biomagnify in aquatic food webs?”, then the uncertainty analysis should focus on the quality, sufficiency and consistency of metrics that will have the most impact on the BMF and should also include data variation (or lack thereof if only a single study or data point is available). In this IATA it is suggested that the uncertainty analysis consist of a qualitative categorical approach (e.g., very low, low, moderate, high, very high) to assess the level of uncertainty given that it is not likely possible to use quantitative approaches in all situations for all evidence. However, this IATA does not discourage quantitative approaches, but notes that the power of such approaches may not improve on expert judgement.

Figure 7. A general figure for the application of the Bioaccumulation IATA



Note: The box for lines of evidence (LoE) generally progresses from less resource intensive data generation (top) to more resource intensive data generation (bottom). The framework can be used in a tiered manner but is intended to be iterative, hence the dashed line from the LoE listing. The tiered progression in the LoE box is generally recommended for addressing uncertainty in decision-making within this IATA but it is not intended to be prescriptive.
Source: (Arnot 2023)

The above uncertainty discussion pertains to evidence that is available for the IATA. Unknowns or true uncertainty propagated from gaps in evidence (e.g., lack of *in vivo* bioaccumulation data) become remaining or residual uncertainties that should be communicated along with IATA outcomes during the evidence integration and reporting step (**Section 7**) noting that the impact of remaining uncertainties on the hypothesis question posed in the problem formulation will vary from not significant to very significant depending on the decision context.

6.3 Reporting the IATA Results

The final, perhaps the most critical step of the IATA, involves reporting outcomes from the data integration including remaining uncertainties. Data integration can be performed using various user defined approaches, but this bioaccumulation IATA should include the following key elements:

- The relevant LoE examined to address the problem statement(s) or all evidence if this is required for transparency purposes;
- An examination of data coherence, that is, how well the various lines of evidence corroborate each other and with the problem statement(s);

- A process that involves assigning weight or preference to individual lines of evidence in order to be able to provide an overall strength of evidence (i.e., strength of inference) to address the problem statement(s);
- An assessment of the strength of evidence to determine if an answer to the problem statement(s) can be reached using the available evidence. If the available evidence does not support the problem statement(s), alternative explanations should be described including key data and knowledge gaps;
- An assessment of the sensitivity of the problem statement(s) to remaining or residual uncertainties (i.e., those not accounted for during the data evaluation and evidence weighing) generated for example through data gaps.

Reporting of the above elements provides transparency to the IATA allowing reviewers to trace how bioaccumulation evidence gathered and evaluated under this IATA was used or not used to reach a conclusion supporting the problem statement(s) or that an alternative explanation is required.

7 Application of IATA

To illustrate examples of the B IATA, the freeware Bioaccumulation Assessment Tool (BAT) and Bioaccumulation Evaluation Tool (BET) are used because they can aid users with implementing the concepts of this IATA. Some BCF QSAR models were also included to illustrate how multiple data sources can be applied in the IATA. The BAT and BET were developed with a WoE approach with concepts similar to OECD 2019 WoE guidance but do not follow this guidance exactly, owing to the fact that their development preceded the 2019 OECD WoE guidance. Some OECD WoE concepts explained in **Sections 5** and **6** were accordingly adapted and explained for the case studies. The IATA data elements described in **Sections 1-6** can be input into the tools directly to allow for integration and evaluation of bioaccumulation data used in the IATA. In the case studies the quantitative data reliability criteria and scoring methods from the B assessment guidance, i.e., published DETs (Arnot 2023), were used to obtain reliability scores for various LoE.

Three examples are provided that provide a spectrum of some possible applications of the IATA with different levels of data available. **These case studies are illustrative examples and should not be interpreted as official regulatory decisions made by the authoring member countries, because any jurisdiction may:**

1) **Select additional data that are not included in the case study, and/or**
2) **Reach a different conclusion about the reliability or weighing of one or more individual studies, based on expert judgment and regulatory context.**

These case studies highlight the flexibility and breadth of the context of applicability for the IATA. They illustrate the utility of the IATA as a transparent, evidence driven evaluation that can be modulated to reflect regional or situational priorities and guidance.

7.1 Data Poor Example – Dicyclohexylphthalate (DCHP)

7.1.1 Purpose & Hypothesis (Problem Formulation) - DCHP

This case example demonstrates how this B IATA can be used for a very “data poor” chemical with no available measured bioaccumulation or biotransformation studies available. This example is conducted for dicyclohexyl phthalate (DCHP; CAS RN 84-61-7). DCHP is a plasticizer used in adhesives and plastic and rubber products and resins and is a granular solid at room temperature. The example is provided to address the following two problem statements:

1. Is there sufficient evidence to support the conclusion that DCHP is bioaccumulative in fish (i.e., bioaccumulation relative to water) using the “B” and “vB” criteria thresholds of 1000 and 5000, respectively? [Problem statement 1]

2. Is there sufficient evidence to support the conclusion that DCHP biomagnifies in aquatic or terrestrial food webs using the recommended (Burkhard 2012) criterion of 1 kg-lw/kg-lw for neutral hydrophobic organic chemicals? [Problem statement 2]

To address these problem statements, the steps outlined in **Sections 4** (data gathering), **5** (data evaluation) and **6** (data integration and reporting) are followed. To help address the needs of each step, reference is made to the freely available US EPA CompTox Dashboard (https://www.epa.gov/chemical-research/comptox-chemicals-dashboard), the Bioaccumulation Evaluation Tool (BET) implemented in EAS-E Suite (www.eas-e-suite.com), other sources (e.g., EPI Suite (US EPA 2011)), and general B assessment guidance published by Arnot and colleagues (Arnot 2023).

As stated in **Section 2.4**, there is no current consensus in the scientific and regulatory communities for relevance scoring for the various bioaccumulation metrics. Although somewhat subjective and variable, it is critical that relevance scoring is communicated in a clear and transparent manner. For this example, we have assigned the relevance criteria in **Table 10** to the various LoE (0 = least relevant; 5 = most relevant).

For problem statement 1, relevance scores reflect concern for potential bioaccumulation in fish relative to water concentrations resulting from uptake from all exposure sources (i.e., via the gills, diet and dermal contact) given that both uptake from water and the diet can be expected for this hydrophobic substance. Laboratory studies are given higher overall relevance than *in silico* results. The lower scores are indicative of a lower relevance given to non-fish species (invertebrates) resulting from little or no regulatory precedence as sentinel bioaccumulation species and scientific uncertainty with bioaccumulation metrics measured in such species (e.g., uptake rates, metabolic capacity). The relevance of invertebrate data is context dependent and may be more relevant in some jurisdictions.

For problem statement 2, relevance scores reflect concern for potential biomagnification in aquatic and terrestrial food webs. Therefore, LoE that provide specific evidence of biomagnification (e.g., BMF and TMF) are given high relevancy scores, with empirical data given higher scores than *in silico* models.

Table 10. Relevance scores assigned a priori to each LoE for DCHP

Problem stmt.	LoE description	Organism type	Relevance Score (0 = least; 5 = most)	Relevance Score Description
1	*In silico* lab BCF	Fish	4	Focus on fish; *in silico* lower score than *in vivo*
	In silico BAF	Fish	4	Focus on fish; *in silico* lower score than *in vivo*
	In silico BAF	Invert	0	Not relevant for this specific example; could be in other jurisdictions; higher uncertainty
	In silico lab BMF	Fish	0	Criteria specific to BCF / BAF
	In silico lab BMF	Rat	0	Focus on fish
	In silico field BMF	Various	0	Criteria specific to BCF / BAF
	In vivo lab BCF	Fish	5	Focus on fish; *in vivo* higher score than *in silico*
	In vivo lab BCF	Invert	0	Not relevant for this specific example; could be in other jurisdictions; higher uncertainty
	In vivo lab BMF	Fish	0	Not relevant for this specific example; could be in other jurisdictions; higher uncertainty
	In vivo field BAF	Various	5	Focus on fish; *in vivo* higher score than *in silico*
	In vivo field BMF	Various	0	Criteria specific to BCF / BAF
	In vivo field TMF	Various	0	Criteria specific to BCF / BAF
2	*In silico* lab BCF	Fish	0	Not relevant for biomagnification
	In silico BAF	Fish	0	Not relevant for biomagnification
	In silico BAF	Invert	0	Not relevant for biomagnification
	In silico lab BMF	Rat	4	Specific biomagnification metric; *in silico* lower score than *in vivo*
	In silico lab BMF	Fish	4	Specific biomagnification metric; *in silico* lower score than *in vivo*
	In silico field BMF	Various	4	Specific biomagnification metric; *in silico* lower score than *in vivo*
	In vivo lab BCF	Fish	0	Not relevant for biomagnification
	In vivo lab BCF	Invert	0	Not relevant for biomagnification
	In vivo lab BMF	Fish	5	Specific biomagnification metric; *in vivo* higher score than *in silico*
	In vivo field BAF	Various	0	Not relevant for biomagnification
	In vivo field BMF	Various	5	Specific biomagnification metric; *in vivo* higher score than *in silico*
	In vivo field TMF	Various	5	Specific biomagnification metric; *in vivo* higher score than *in silico*

7.1.2 Approach - DCHP

7.1.2.1 Physical-chemical Properties - DCHP

Following the problem formulation phase, the next step is to gather relevant physical-chemical property information for DCHP, starting with the critical information required to apply available BCF, BAF and BMF prediction models for aquatic and terrestrial species, respectively. Because this DCHP is a neutral hydrophobic organic chemical the MW, K_{OW} and K_{OA} are needed for the problem statements. Not

considered here is the option to obtain biological partitioning data to possibly consider the uncertainty of using octanol as a surrogate for biological partitioning in the models (traditionally, the assumption of octanol as a surrogate for biological partitioning is common and is indeed explicit in all current B screening criteria using K_{OW}). DCHP is a neutral chemical, so pKa is not needed. **Table 11** provides a summary of the physical-chemical property information for DCHP obtained from various sources via the USEPA CompTox Dashboard and EAS-E Suite. It is important to note that there is currently no standardized guidance to critically evaluate the physical-chemical property information used in this case example. However, Li et al. (Li 2022) should be consulted to guide the appropriate selection and preliminary evaluation of physical-chemical property information. Additionally, as an interim, for QSAR and Poly-Parameter Linear Free Energy Relationship (PPLFER) predictions of chemical properties, the generic DET for QSAR predictions shown in **Table 4** can be considered. For illustrative purposes, the modeled values that were within the applicability domain of the specified models (according to EAS-E Suite and/or the USEPA CompTox Dashboard) are included in **Table 11**. All model results are for models where DCHP was within the applicability domain. By arranging the property information and clearly noting which value(s) were used in the assessment, transparency is ensured and a rationale for why a particular value was or was not used can be easily provided.

Table 11. Experimental and estimated physical-chemical properties for DCHP

Property (value used)	Value	Units	Source
MW (330.42)	330.42	g/mol	
log K_{OW} (6.18)	6.18	Unitless	Average (consensus) of KOWWIN_v1.69-AFC & IFSQSAR LogKOW-ppLLFER_v1.0
	6.20		KOWWIN_v1.69-AFC
	6.17		IFSQSAR LogKOW-ppLLFER_v1.0
	5.83		OPERA logP-KNN
Log K_{OA} (11.8)	11.8	Unitless	Average (consensus) of KOAWIN_v1.11-Exp-based calculation & IFSQSAR LogKOA-ppLFER_v1.0
	11.6		KOAWIN_v1.11-Exp-based calculation
	12.0		IFSQSAR LogKOA-ppLFER_v1.0
	10.7		KOAWIN_v1.11-KowWin/HenryWin
	10.7		OPERA logKOA-KNN

Note: Values selected for this assessment are noted in parentheses in the first column. All values are model estimates unless otherwise noted.
Model access date July 2023

Box 1. Initial Evaluation

This case study is intended to reflect a scenario using "B" and "vB" criteria thresholds of 1000 and 5000 L/kg-ww where physical-chemical screening criteria might not be explicitly included. However, following the draft guidance shown in Figure 7, initial collection of physical-chemical properties for DCHP indicates that this chemical would need further assessment for bioaccumulation in aquatic and terrestrial systems. Based on this information, there is not sufficient confidence to make a decision, and we progress to the next step of the workflow.

7.1.2.2 Biotransformation - DCHP

It is well-documented that an organism's capacity to biotransform a chemical can have a significant impact on bioaccumulation potential for hydrophobic organic chemicals (Nichols 2007; Arnot 2008b; McLachlan 2011). Estimates of whole-body biotransformation can be obtained via *in silico*, *in vitro*, and *in vivo* models, and these values can then be utilized within existing bioaccumulation models. For DCHP, no *in vivo* or *in vitro* data are available. However, whole-body level biotransformation half-lives (HL_B) for mammals (humans) and fish can be predicted *in silico*. This HL_B information can then be used to parameterize toxicokinetic and BCF, BAF and BMF models. Bioaccumulation toxicokinetic models in the BET module in the EAS-E Suite platform can readily be applied where one can select which biotransformation parameters to use based on an evaluation. Results of various HL_B-QSAR predictions using EAS-E Suite and the USEPA CompTox Chemical Dashboard are summarized in **Table 12**. All results are for models where DCHP was within the applicability domain. Other HL_B-QSARs could also be considered, e.g., from EPI Suite.

Table 12. Summary of the whole-body biotransformation half-life (HL_B) information for DCHP

Metric	Species	Value (h)	Source / notes
Half-life (whole-body) *in silico prediction*	Human (@ 70 kg)	3.24	IFSQSAR hhlb-IFS/QSARINS HumanB1-Molecular descriptors/QSARINS HumanB2-Molecular descriptors/QSARINS HumanB3-Molecular descriptors/QSARINS HumanB4-Molecular descriptors (consensus of 5 values) _v1.0
		0.82	IFSQSAR hhlb-IFS_v1.0
		5.5	QSARINS HumanB1-Molecular descriptors
		3.01	QSARINS HumanB2-Molecular descriptors
		3.92	QSARINS HumanB3-Molecular descriptors
		6.75	QSARINS HumanB4-Molecular descriptors
	Fish (@ 0.01 kg)	14.24	IFSQSAR fhlb-IFS/QSARINS FishM1-Molecular descriptors/QSARINS FishM2-Molecular descriptors/QSARINS FishM3-Molecular descriptors (consensus of 4 values) _v1.0
		20.4	IFSQSAR fhlb-IFS_v1.0
		11.91	QSARINS FishM1-Molecular descriptors
		14.86	QSARINS FishM2-Molecular descriptors
		11.4	QSARINS FishM3-Molecular descriptors
		12.6	OPERA 2.6

Note: Model access date July 2023.

Box 2. Evaluation

For fish, the results from 5 in silico half-life predictions that are all within the applicability domain are very consistent. For the purposes of this example, we will use the consensus (average) of the 4 modeled values available within EAS-E Suite for fish (14 h) to progress to the next phase of the workflow. For mammals, the results from 5 in silico half-life predictions that are all within the applicability domain are generally consistent, with one value (IFSQSAR hhlb-IFS) a bit lower. The decision was made to use the consensus of those 5 values available within EAS-E Suite for humans (3 h) to progress to the next phase of the workflow.

7.1.2.3 *In silico B Predictions - DCHP*

The next step after obtaining physical-chemical property and biotransformation rate information is to utilize existing models to predict B metrics to address the problem statements. There are no empirical B data available for DCHP, so model predictions are the only methods currently available to obtain LoE for B assessment. Bioaccumulation models that provide B metrics (LoE) to address the problem statements can be considered. In this case example we consult the BCFBAF model in EPI Suite (US EPA 2011), the OPERA model in the US EPA CompTox Dashboard, and the BET in EAS-E Suite ver.0.9. For the purpose of this evaluation, the consensus predictions for $HL_{B,N}$ of 14 h and 3 h for fish and mammals were used, respectively. These values, along with MW, K_{OW} and K_{OA} in **Table 11** were used to parameterize the BET to obtain problem relevant lab and field B metrics.

A summary of the *in silico* B metric predictions that can be used to address problem statements 1 and 2 is provided in **Tables 13-15**. The BCF and BAF data in **Table 13** can be used to address problem statement 1 and **Tables 14 and 15** can be used to address problem statement 2.

Table 13. In silico laboratory BCF and field BAF model predictions – DCHP

Metric	Value	Units	Model
In silico fish BCF 1	162	L/kg-ww	OPERA 2.6
In silico fish BCF 2	517	L/kg-ww	BET ver. 0.9
In silico fish BCF 3	5750	L/kg-ww	BCFBAF – EPI Suite (regression-based estimate)
In silico fish BCF 4	134.9	L/kg-ww	BCFBAF – EPI Suite (Arnot & Gobas; upper TL)
In silico fish BCF 5	185.0	L/kg-ww	BCFBAF – EPI Suite (Arnot & Gobas; mid TL)
In silico fish BCF 6	203.8	L/kg-ww	BCFBAF – EPI Suite (Arnot & Gobas; lower TL)
In silico fish BAF 1	136.9	L/kg-ww	BCFBAF – EPI Suite (Arnot & Gobas; upper TL)
In silico fish BAF 2	265.7	L/kg-ww	BCFBAF – EPI Suite (Arnot & Gobas; mid TL)
In silico fish BAF 3	794.7	L/kg-ww	BCFBAF – EPI Suite (Arnot & Gobas; lower TL)
In silico fish BAF 4	606	L/kg-ww	BET ver. 0.9 (plank fish)
In silico fish BAF 5	599	L/kg-ww	BET ver. 0.9 (benthic fish)
In silico fish BAF 6	725	L/kg-ww	BET ver. 0.9 (omniv fish)
In silico fish BAF 7	1010	L/kg-ww	BET ver. 0.9 (pisciv fish)

Note: Model access date July 2023

The *in silico* BET (and BAT) model BMF_L predictions for a representative lab fish and lab rat are shown in **Table 14.** The fish model simulates an OECD 305 bioaccumulation test for dietary exposures and the subsequent BMF_L metric in a typical 0.01 kg, 5% lipid content fish. The rat model is conceptually similar and considered representative of a generic laboratory rat used in an OECD 417 Testing Guideline experiment. The input parameters (K_{OW}, K_{OA} and $HL_{B,N}$) used in these simulations are considered to be of high quality (reliability) and the ensuing BMF predictions are considered acceptable. **Table 15** shows the *in silico* BET (and BAT) model BMF_L predictions for representative aquatic and terrestrial food webs. The BMFs for fish in the field are higher than the BMFs in the laboratory because the organisms in the field are co-exposed to the chemical in the water as well as their diet.

Table 14. In silico BMF_L model predictions for a representative lab fish and lab rat – DCHP

Metric	Value	Units	Model
In silico fish BMF 1	0.012	kg-lw/kg-lw	BET ver. 0.9
In silico rat BMF 1	0.015	kg-lw/kg-lw	BET ver. 0.9

Note: Model access date July 2023

Table 15. In silico BMF_L model predictions for representative aquatic and terrestrial food webs – DCHP

Metric	Organism	Value	Units	Model
In silico field BMF 1	Planktivorous fish	0.07	kg-diet/kg-org [lw]	BET ver. 0.9
In silico field BMF 2	Benthic fish	0.17	kg-diet/kg-org [lw]	BET ver. 0.9
In silico field BMF 3	Omnivorous fish	0.22	kg-diet/kg-org [lw]	BET ver. 0.9
In silico field BMF 4	Piscivorous fish	0.55	kg-diet/kg-org [lw]	BET ver. 0.9
In silico field BMF 5	Aquatic mammal	0.01	kg-diet/kg-org [lw]	BET ver. 0.9
In silico field BMF 6	Small rodent	0.008	kg-diet/kg-org [lw]	BET ver. 0.9
In silico field BMF 7	Terrestrial herbivore	0.002	kg-diet/kg-org [lw]	BET ver. 0.9
In silico field BMF 8	Terrestrial carnivore	0.01	kg-diet/kg-org [lw]	BET ver. 0.9
In silico field BMF 9	Avian passerine	0.02	kg-diet/kg-org [lw]	BET ver. 0.9
In silico field BMF 10	Avian piscivorous	0.02	kg-diet/kg-org [lw]	BET ver. 0.9
In silico field BMF 11	Avian terrestrial	0.02	kg-diet/kg-org [lw]	BET ver. 0.9

Note: Model access date July 2023

Box 3. Evaluation

Problem Statement 1: *Five of the six in silico BCF model predictions indicate that DCHP is not likely to bioaccumulate in fish. One model BCF estimate (EPI Suite regression-based estimate) is above the "vB" threshold of 5000 L/kg-ww for fish and one BAF estimate is above the "B" threshold of 1000 L/kg-ww for fish. The high prediction for the BCF value from the EPI Suite regression-based method can be explained because regression-based QSAR models, like the one in BCFBAF, do not include chemical-specific values for biotransformation rates. The multiple-linear regression QSARs are fit to average values determined from the fragments included in the model and the chemical. The available data from multiple HL_B-QSAR predictions that are chemical structure specific indicate that DCHP is biotransformed relatively quickly in fish. The efficient biotransformation is not unexpected as esters are often biotransformed relatively well in biota. Because no further data for DCHP are available, the decision could be made at this stage to utilize the general concordance of the various in silico model estimates for aquatic BCF and BAF to make a conclusion.*

Problem Statement 2: *All of the in silico lab and field BMF model predictions indicate that DCHP does not biomagnify in representative aquatic and terrestrial laboratory experiments and representative food web species.*

7.1.3 Data Evaluation and Weighing - DCHP

The next phase of the B IATA includes data evaluation and data weighing. Data evaluation uses the DETs described in **Section 5.0** to critically evaluate each LoE for reliability. The data weighing step assigns a weight to each LoE by combining the relevance score that was assigned *a priori* within the problem formulation phase with the results from the data evaluation phase.

7.1.3.1 Data Evaluation - DCHP

DCHP is considered a very data poor chemical requiring that all bioaccumulation data be generated using *in silico* approaches (i.e., inputs to models and outputs from models). DCHP is a relatively simple neutral organic structure (cyclic ester phthalate) and as such amenable to *in silico* predictions well within both the model parameter domain (physical-chemical property) and structural domains of all models used in **Tables 11 - 15**. Several cyclic and linear ester structures are contained within the physical-chemical, biotransformation and bioaccumulation model training sets giving greater confidence with model input and output. Consequently, although evidence is limited to *in silico* predictions for DCHP, the model results were considered of high quality and consistent with empirical knowledge of the relatively quick biotransformation of phthalate esters to mono-esters and di-carboxylic acids which then become suitable for urinary elimination. Biotransformation and biomagnification model results support this conclusion. An exception is noted in **Section 7.1.2.2** for the regression-based EPI Suite BCF model where biotransformation is not explicitly considered for this chemical structure and log K_{OW} drives a linear prediction with BCF.

7.1.3.2 Weighing LoE

Summaries of the *in silico* lines of evidence used to evaluate DCHP according to each of the problem statements are shown in **Tables 16 - 17**. Note that only the LoE for which information was available and relevance was >0 are reported in the tables. In the summary section (worksheet) of the BAT there is a summary of the detailed information related to the reliability of each LoE which can be transparently documented and communicated. The BAT worksheet scores were used to help understand data quality and associated uncertainty. The reliability scores in **Tables 16 - 17** thus holistically reflect these WoE concepts. Reliability in this case study is thus a measure of the confidence with each line of evidence to address the problem statement using “B” and “vB” criteria thresholds of 1000 and 5000 L/kg-ww (i.e., to make decision). In this example, a reliability scale is proposed for the *in silico* models based on existing knowledge. Future ongoing work will focus on further defining data quality scores for these models. Users may also define their own scales. The weight given to each LoE is a function of relevance and reliability. In this example, we use three non-numerical bins of “low”, “moderate”, and “high” to convey the weight of each LoE. Scores of relevance and reliability were combined together and indicate an overall score (9) close to the maximum achievable and therefore a judgement of high weight was assigned to the *in silico* information.

Table 16. Summary of LoE to support problem statement 1 for DCHP

LoE	"B" cat.	Relevance	Data Reliability Score	LoE Weight
In silico fish BCF 1	nB	4	5	High
In silico fish BCF 2	nB	4	5	High
In silico fish BCF 3	vB	4	5	High
In silico fish BCF 4	nB	4	5	High
In silico fish BCF 5	nB	4	5	High
In silico fish BCF 6	nB	4	5	High
In silico fish BAF 1	nB	4	5	High
In silico fish BAF 2	nB	4	5	High
In silico fish BAF 3	nB	4	5	High
In silico fish BAF 4	nB	4	5	High
In silico fish BAF 5	nB	4	5	High
In silico fish BAF 6	nB	4	5	High
In silico fish BAF 7	B	4	5	High

Table 17. Summary of LoE to support problem statement 2 for DCHP

LoE	"B" cat.	Relevance	Data Reliability Score	LoE Weight
In silico fish BMF 1	nB	4	5	High
In silico rat BMF 1	nB	4	5	High
In silico field BMF 1	nB	4	5	High
In silico field BMF 2	nB	4	5	High
In silico field BMF 3	nB	4	5	High
In silico field BMF 4	nB	4	5	High
In silico field BMF 5	nB	4	5	High
In silico field BMF 6	nB	4	5	High
In silico field BMF 7	nB	4	5	High
In silico field BMF 8	nB	4	5	High
In silico field BMF 9	nB	4	5	High
In silico field BMF 10	nB	4	5	High
In silico field BMF 11	nB	4	5	High
In silico field BMF 12	nB	4	5	High

7.1.4 Bioaccumulation Data Integration and Reporting – DCHP

7.1.4.1 Strength of Evidence and Residual Uncertainty

A high-level summary of the various available LoE relevant to the problem statements for DCHP and the corresponding nB, B, and vB classifications of each LoE per the criteria thresholds as defined in each problem statement are shown in **Table 18.** Clearly, in this DCHP example, model results are consistent with low variation across all properties and endpoints predicted as evidenced in **Tables 11 – 15** for this in-domain compound. Overall uncertainty of the *in silico* predictions was therefore judged to be low. Lack of empirical data points to a potentially significant data gap for DCHP in this IATA example. Consequently,

reliance on *in silico* data alone may or may not be sufficient for regulatory conclusions depending on context. A key factor in this context question is whether a regulatory regime allows for secondary steps to be performed (i.e., as a tiered assessment process).

Figure 8 provides a high-level summary of the existing information for DCHP related to problem statement 1 (bioaccumulation). The WoE suggests that DCHP is "nB" based on the available *in silico* information. Depending on the specific decision context and the required level of confidence, the evidence could be sufficient to arrive at a regulatory conclusion for bioaccumulation (e.g., prioritization for risk assessment). Other decisions could involve obtaining more model predictions (from additional models or approaches), or the decision could be that a level of confidence for a regulatory decision is insufficient, and additional data are required to support or refute the information provided by the current *in silico* predictions (e.g., an OECD TG 305 BCF). Following the B IATA workflow and application guidance (**Figures 3 and 7**), a pragmatic next step could be to obtain *in vitro* biotransformation rate data for DCHP in fish and apply an IVIVE model to compare with the *in silico* data, and if deemed necessary, conduct additional BET-type model simulations with refined HL_B input.

Table 18. Summary of LoE for DCHP and corresponding "B" classifications per criteria thresholds as specified in each problem statement to indicate strength of evidence

Problem Statement	LoE description	Organism type	# LoE	Total # LoE	%nB	%B	%vB
1	*In silico* lab BCF	Fish	6	13	85%	7.5%	7.5%
	In silico BAF	Fish	7				
2	*In silico* lab BMF	Fish	1	17	100%	0%	N/A
	In silico lab BMF	Rat	1				
	In silico field BMF	Various	15				

Note: Only data considered reliable with relevancy scores >0 are included within this table.

7.1.5 Summary - DCHP

DCHP is a very "data poor" chemical, and therefore evaluation of the problem statements for bioaccumulation and biomagnification are limited to information from *in silico* modeling approaches. However, despite the lack of available empirical data, the *in silico* predictions are relevant, of high quality, and relatively consistent across multiple LoE to address the two problem statements. As a data poor example substance, the coherence of various types of bioaccumulation evidence for DCHP cannot be addressed here. Lack of the ability to assess data coherence (e.g., concordance with *in vivo* data) may or may not play a role in assigning the overall strength of the available evidence. Again, this is decision context specific as discussed below.

A summary of the existing information for DCHP related to problem statement 2 (biomagnification) is shown in **Table 18.** The WoE suggests that DCHP does not biomagnify in aquatic food webs based on the available *in silico* information. Depending on the specific decision context and acceptable level of confidence the decision could be made to ask for additional data to support or refute the information provided by the current *in silico* predictions. As outlined in the IATA workflow, the next pragmatic step would be to obtain some *in vitro* biotransformation rate studies following OECD TG 319 A/B (OECD 2018b, 2018c) and incorporating that new data with the in silico HL_B predictions into a BMF model, i.e., refine the input parameters used in the BET model calculations or to conduct a laboratory biomagnification study (e.g., an OECD TG 305 BMF).

Figure 8. Summary of available evidence related to problem statement 1 for DCHP

7.2 Data Poor Example – 1,2-Dibromo-4-(1,2-dibromoethyl)cyclohexane (TBECH)

7.2.1 Purpose & Hypothesis (Problem Formulation) – TBECH

The purpose of this example is to demonstrate how this IATA can be utilized in a rather "data poor" scenario to transparently evaluate all data consistently and objectively and identify uncertainties and gaps that should be addressed and their impact on decision-making. 1,2-Dibromo-4-(1,2-dibromoethyl)cyclohexane (TBECH; CAS 3322-93-8), also known as tetrabromoethylcyclohexane (TBECH), is used primarily as an additive flame retardant and marketed as Saytex BCL-462. TBECH is primarily used in expandable polystyrene beads (used for thermal insulation in housing) and it is also used as a flame retardant for extruded polystyrene foam and for adhesives in fabric and vinyl lamination, electrical cable coatings, high-impact plastic parts of appliances and some construction materials (US EPA 1984). Production of TBECH was reported to be between 4 and 225 metric tonnes in 2002 (Arsenault 2008). It belongs to the group of novel brominated flame retardants (BFRs) which have been developed as replacement for traditional BFRs, such as polybrominated diphenyl ethers, polybrominated biphenyl and hexabromocyclododecanes which have been banned or strictly phased out due to their PBT properties (Hou 2021). The example can be addressed using the following two problem statements:

1. Is there sufficient evidence to support the conclusion that TBECH is bioaccumulative in the aquatic environment (referenced to water) using the "B" and "vB" criteria thresholds of 2000 L/kg-ww and 5000 L/kg-ww, respectively? [Problem statement 1]

2. Is there sufficient evidence to support the conclusion that TBECH biomagnifies in aquatic or terrestrial food webs using the recommended (Burkhard 2012) criterion of 1 kg-lw/kg-lw for neutral hydrophobic organic chemicals? [Problem statement 2]

To address these problem statements, the steps outlined in **Sections 4** (data gathering), **5** (data evaluation) and **6** (data integration and reporting) are followed. To help address the needs of each step, reference is made to the freely available US EPA CompTox Dashboard (https://www.epa.gov/chemical-research/comptox-chemicals-dashboard), the Bioaccumulation Estimation Tool (BET) implemented in EAS-E Suite (www.eas-e-suite.com), other sources (e.g., EPI Suite (US EPA 2011)), and general B assessment guidance published by Arnot and colleagues (Arnot 2023).

As stated in **Section 2.4**, there is no current consensus in the scientific and regulatory communities for relevance scoring for the various bioaccumulation metrics. Although somewhat subjective and variable, it is critical that relevance scoring is communicated in a clear and transparent manner. For this example, we have assigned the relevance criteria in **Table 19** to the various LoE (0 = least relevant; 5 = most relevant).

For problem statement 1, relevance scores reflect concern for potential bioaccumulation in fish relative to water concentrations resulting from uptake from all exposure sources (i.e., via the gills, diet and dermal contact) given that both uptake from water and the diet can be expected for this hydrophobic substance. Laboratory studies are given higher overall relevance than *in silico* results. The lower scores are indicative of a lower relevance given to non-fish species (invertebrates) resulting from little or no regulatory precedence as sentinel bioaccumulation species and scientific uncertainty with bioaccumulation metrics measured in such species (e.g., uptake rates, metabolic capacity). The relevance of invertebrate data is context dependent and may be more relevant in some jurisdictions. In this example invertebrates are considered relevant to this regulatory context, whereas in the DCHP example, they were considered not relevant due to the regulatory context.

For problem statement 2, relevance scores reflect concern for potential biomagnification in aquatic and terrestrial food webs. Therefore, LoE that provide specific evidence of biomagnification (e.g., BMF and TMF) are given high relevancy scores, with empirical data given higher scores than *in silico* models.

Table 19. Relevance scores assigned a priori to each LoE for TBECH

Problem stmt.	LoE description	Organism type	Relevance Score (0 = least; 5 = most)	Relevance Score Description
1	*In silico* lab BCF	Fish	4	Focus on aquatic; *in silico* lower score than *in vivo*
	In silico BAF	Fish	4	Focus on aquatic; *in silico* lower score than *in vivo*
	In silico BAF	Invert	3	Inverts relevant for this specific example; higher uncertainty; *in silico* lower score than *in vivo*
	In silico lab BMF	Fish	0	Criteria specific to BCF / BAF
	In silico lab BMF	Rat	0	Focus on aquatic; criteria specific to BCF / BAF
	In silico field BMF	Various	0	Criteria specific to BCF / BAF
	In vivo lab BCF	Fish	5	Focus on aquatic; *in vivo* higher score than *in silico*
	In vivo lab BCF	Invert	4	Inverts relevant for this specific example; higher uncertainty; *in vivo* higher score than *in silico*
	In vivo lab BMF	Fish	0	Criteria specific to BCF / BAF
	In vivo field BAF	Various	5	Focus on aquatic; *in vivo* higher score than *in silico*
	In vivo field BMF	Various	0	Criteria specific to BCF / BAF
	In vivo field TMF	Various	0	Criteria specific to BCF / BAF
2	*In silico* lab BCF	Fish	0	Not relevant for biomagnification
	In silico BAF	Fish	0	Not relevant for biomagnification
	In silico BAF	Invert	0	Not relevant for biomagnification
	In silico lab BMF	Fish	4	Specific biomagnification metric; *in silico* lower score than *in vivo*
	In silico lab BMF	Rat	4	Specific biomagnification metric; *in silico* lower score than *in vivo*
	In silico field BMF	Various	4	Specific biomagnification metric; *in silico* lower score than *in vivo*
	In vivo lab BCF	Fish	0	Not relevant for biomagnification
	In vivo lab BCF	Invert	0	Not relevant for biomagnification
	In vivo lab BMF	Fish	5	Specific biomagnification metric; *in vivo* higher score than *in silico*
	In vivo field BAF	Various	0	Not relevant for biomagnification
	In vivo field BMF	Various	5	Specific biomagnification metric; *in vivo* higher score than *in silico*
	In vivo field TMF	Various	5	Specific biomagnification metric; *in vivo* higher score than *in silico*

7.2.2 Approach - TBECH

7.2.2.1 Physical-chemical Properties - TBECH

Following the problem formulation phase, the next step is to gather relevant physical-chemical property information for TBECH, starting with the critical information required to apply available BCF, BAF and BMF prediction models for aquatic and terrestrial species, respectively. Because TBECH is a neutral hydrophobic organic chemical the MW, K_{OW} and K_{OA} are needed for the problem statements. Not considered here is the option to obtain biological partitioning data to possibly consider the uncertainty of

using octanol as a surrogate for biological partitioning in the models (traditionally, the assumption of octanol as a surrogate for biological partitioning is common and is indeed explicit in all current B screening criteria using K_{OW}). TBECH is a neutral chemical, so pKa is not needed. **Table 20** provides a summary of the physical-chemical property information for TBECH obtained from various sources via the USEPA CompTox Dashboard and EAS-E Suite. It is important to note that there is currently no standardized guidance to critically evaluate the physical-chemical property information used in this case example. However, Li et al., (Li 2022) should be consulted to guide the appropriate selection and preliminary evaluation of physical-chemical property information. Additionally, as an interim, for QSAR and Poly-Parameter Linear Free Energy Relationship (PPLFER) predictions of chemical properties, the generic DET for QSAR predictions shown in **Table 4** can be considered. For illustrative purposes, the modeled values that were within the applicability domain of the specified models (according to EAS-E Suite and/or the USEPA CompTox Dashboard) are included in **Table 20**. By arranging the property information and clearly noting which value(s) were used in the assessment, transparency is ensured and a rationale for why a particular value was or was not used can be easily provided.

A technical mixture of TBECH consists largely of two diastereoisomers, rac-(1R,2R)-1,2-dibromo-(4S)-4-((1S)-1,2-dibromoethyl)cyclohexane (α-TBECH) and rac-(1R,2R)-1,2-dibromo-(4S)-4-((1R)-1,2-dibromoethyl)cyclohexane (β-TBECH) in an approximate 1:1 ratio. The other two possible isomers, γ-TBECH and δ-TBECH were not detected in a technical mixture. Although technical TBECH does not contain the γ- and δ-isomers, they may still be relevant environmental contaminants since manufacturing utilizes thermal processes which may induce their formation (Arsenault 2008). The different diastereomers may exhibit different environmental behaviors and biological effects.

A summary of predicted physical chem parameters are shown in **Table 20.** Although there are different isomers for TBECH, *in silico* (QSAR) models cannot typically differentiate between chemical properties for isomers.

Table 20. Estimated physical-chemical properties for TBECH

Property (value used)	Value	Units	Source
MW (427.8)	427.8	g/mol	
log K_{OW} (5.33)	5.33	Unitless	KOWWIN_v1.69-AFC & IFSQSAR LogKOW-ppLLFER_v1.0 (consensus of 2 values) _v1.0
	5.24		KOWWIN_v1.69-AFC (outside of AD)
	5.43		IFSQSAR LogKOW-ppLLFER_v1.0
	4.44		ACD/Labs
	4.61		ACD/Labs Consensus
	5.15		OPERA 2.6 (outside AD)
	5.24		EPI Suite (KOWWIN v1.68)
Log K_{OA} (8.40)	8.40	Unitless	KOAWIN_v1.11-Exp-based calculation & IFSQSAR LogKOA-ppLFER _v1.0 (consensus of 2 values)
	8.80		IFSQSAR LogKOA-ppLFER_v1.0
	8.01		KOAWIN_v1.11-KowWin/HenryWin
	8.42		OPERA 2.6

Note: Values selected to utilize for this assessment are noted in parentheses in the first column. All values are model estimates unless otherwise noted. *Model access date July 2023.*

Box 4. Initial Evaluation

Based on this initial collection of physical-chemical properties for TBECH, the log K_{OW} is >4.5 and this chemical would need further assessment. Additionally, the log K_{OW} of TBECH is >2 and the log K_{OA} is >5, which also identifies this chemical for further assessment for terrestrial bioaccumulation. For this example, we determine that there is not sufficient confidence to answer either of the problem statements (1 & 2) and therefore proceed to the next step of the workflow.

7.2.2.2 Biotransformation - TBECH

It is well-documented that an organism's capacity to biotransform a chemical can have a significant impact on bioaccumulation potential for hydrophobic organic chemicals (Nichols 2007; Arnot 2008b; McLachlan 2011). Estimates of whole-body biotransformation can be obtained via *in silico*, *in vitro*, and *in vivo* models, and these values can then be utilized within existing bioaccumulation models. This HL_B information can then be used to parameterize toxicokinetic and BCF, BAF and BMF models. Bioaccumulation toxicokinetic models in the BET module in the EAS-E Suite platform can readily be applied where one can select which biotransformation parameters to use based on an evaluation.

Predicted TBECH biotransformation pathways are shown in **Figure 9** and involve hydroxylation, debromination and conjugation with glutathione. *In vitro* biotransformation of TBECH was investigated in liver microsomes from rat (Chu 2012) and human (Nguyen 2017). *In vitro* biotransformation in rat liver microsomes mainly involved hydroxylation of both, a- and β-monomers resulting in mono-and dihydroxy-TBECH and no debromination was observed; however, a rate was not calculated (Chu 2012). Indications of potential cytochrome P450-catalyzed hydroxylation were also found in human liver microsomes, as well

as debromination and α–oxidation (Nguyen 2017). Mono-hydroxylated TBECH was the major metabolite. Estimated *in vitro* intrinsic clearance based on the formation of mono- and dihydroxy metabolites of the TBECH mixture was slower compared to the pure β-TBECH (Nguyen 2017).

Zheng et al (Zheng 2018) determined *in vitro* biotransformation rates in microsomes from three fish species (crucian carp, catfish and yellow-head catfish) as comparison to measured concentrations in different organisms from Lake Taihu, China. *In vitro* intrinsic clearance ranged from 0.011 to 0.027 mL/h/mg protein. However, incubation times (24 h) exceeded 2 hours of incubation recommended for subcellular fractions (OECD 2018c) and the final DMSO concentrations in the assay were ≥1%, i.e. both conditions that result in a critical failure according to the DET criteria (**Table 5**). Additionally, although mentioned in the paper, no data for the heat inactivated controls were shown, thus no statistical comparison to the abiotic decrease can be performed. Therefore, these data were not considered valid for the evaluation process.

Results of various HL_B -QSAR predictions using EAS-E Suite and the USEPA CompTox Chemical Dashboard, as well as the human liver microsome study (Nguyen 2017) are summarized in **Table 21.** All results are for models where TBECH was within the applicability domain.

Figure 9. Predicted biotransformation pathway of TBECH by Catalogic (models accounting for metabolism, non-kinetic) based on information from mammalian models

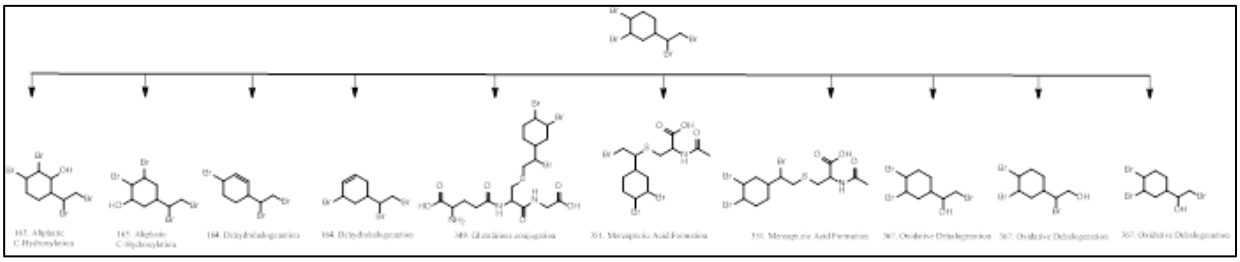


Table 21. Summary of available biotransformation information for TBECH

Metric	Species	Value (h)	Source / notes
Half-life (whole-body) *In silico prediction*	Human (@ 70 kg)	68.84	IFSQSAR hhlb-IFS/QSARINS HumanB1-Molecular descriptors/QSARINS HumanB2-Molecular descriptors/QSARINS HumanB3-Molecular descriptors (Consensus of 4 values) _v1.0
		150.27	IFSQSAR hhlb-IFS_v1.0
		2.16	QSARINS HumanB1-Molecular descriptors
		57.15	QSARINS HumanB2-Molecular descriptors *(outside of AD)*
		1210.6	QSARINS HumanB3-Molecular descriptors *(outside of AD)*
	Fish (@ 0.01 kg)	204.08	IFSQSAR fhlb-IFS/QSARINS FishM1-Molecular descriptors/QSARINS FishM2-Molecular descriptors/QSARINS FishM3-Molecular descriptors (consensus of 4 values) _v1.0
		122.81	IFSQSAR fhlb-IFS_v1.0
		331.89	QSARINS FishM1-Molecular descriptors
		152.41	QSARINS FishM2-Molecular descriptors
		279.25	QSARINS FishM3-Molecular descriptors
		72.48	OPERA 2.6
		40.7	BCFBAF – EPI Suite
Half-life (whole-body) *In vitro*	Human (liver microsomes)	23.07*	Nguyen et al. 2017 (sum of CLs from 6 metabolite pathways)

*Extrapolated to whole-body half-life using IVIVE model in EAS-E suite from in vitro data reported in the original citation
Note: Model access date July 2023

Box 5. Evaluation

For fish, the results from 4 of the 5 in silico half-life predictions for TBECH are relatively consistent. Although there is one in vitro study for fish (three species), the study was deemed unsuitable to use (see above). For the purposes of this example, we will use the consensus of the 4 modeled values available within EAS-E Suite for fish (204 h) as a conservative estimate to progress to the next phase of the workflow. For mammals, the results from 4 in silico half-life predictions are relatively variable. In addition, available in vitro human data from microsomes are available. As a conservative estimate at this stage of the evaluation, the in silico consensus average HL_B across all 4 QSAR prediction models (68.84 h) was used as an estimate of mammalian biotransformation for the next steps within the workflow.

7.2.2.3 In silico B Predictions – TBECH

The next step after obtaining physical-chemical property and biotransformation rate information is to utilize existing models to predict B metrics to address the problem statements. Bioaccumulation models that provide B metrics (LoE) to address the problem statements can be considered. In this case example we consult the BCFBAF model in EPI Suite (US EPA 2011), the OPERA model in the US EPA CompTox Dashboard, and the BET in EAS-E Suite Ver.0.97. For the purpose of this evaluation, the consensus predictions for $HL_{B,N}$ of 204 h and 68.84 h for fish and mammals were used, respectively. These values, along with MW, K_{OW} and K_{OA} in **Table 20** were used to parameterize the BET to obtain problem relevant lab and field B metrics.

Summaries of the *in silico* B metric predictions that can be used to address problem statements 1 and 2 are shown in **Tables 22 – 24**. The BCF and BAF data in **Table 22** can be used to address problem statement 1 and **Tables 23 - 24** can be used to address problem statement 2.

Table 22. In silico BCF and BAF model predictions for TBECH

Metric	Value	Units	Model
In silico fish BCF 1	1380	L/kg-ww	OPERA 2.6
In silico fish BCF 2	5500	L/kg-ww	BET ver. 0.9
In silico fish BCF 3	757	L/kg-ww	TEST
In silico fish BCF 4	1330	L/kg-ww	BCFBAF – EPI Suite (regression-based estimate)
In silico fish BCF 5	659	L/kg-ww	BCFBAF – EPI Suite (Arnot & Gobas; upper TL)
In silico fish BCF 6	872	L/kg-ww	BCFBAF – EPI Suite (Arnot & Gobas; mid TL)
In silico fish BCF 7	943	L/kg-ww	BCFBAF – EPI Suite (Arnot & Gobas; lower TL)
In silico fish BAF 1	676	L/kg-ww	BCFBAF – EPI Suite (Arnot & Gobas; upper TL)
In silico fish BAF 2	1040	L/kg-ww	BCFBAF – EPI Suite (Arnot & Gobas; mid TL)
In silico fish BAF 3	1550	L/kg-ww	BCFBAF – EPI Suite (Arnot & Gobas; lower TL)
In silico fish BAF 4	9310	L/kg-ww	BET ver. 0.9
In silico fish BAF 5	9380	L/kg-ww	BET ver. 0.9
In silico fish BAF 6	12,100	L/kg-ww	BET ver. 0.9
In silico fish BAF 7	17,000	L/kg-ww	BET ver. 0.9
In silico invert BAF 1	7700	L/kg-ww	BET ver. 0.9
In silico invert BAF 2	7270	L/kg-ww	BET ver. 0.9
In silico invert BAF 3	7330	L/kg-ww	BET ver. 0.9

Note: Model access date July 2023

The *in silico* BET (and BAT) model BMF_L predictions for a representative lab fish and lab rat are shown in **Table 23**. The fish model simulates an OECD 305 bioaccumulation test for dietary exposures and the subsequent BMF_L metric in a typical 0.01 kg, 5% lipid content fish. The rat model is conceptually similar and considered representative of a generic laboratory rat used in an OECD 417 TG experiment. The input parameters (K_{OW}, K_{OA} and $HL_{B,N}$) used in these simulations are considered to be of high quality (reliability) and the ensuing BMF predictions are considered acceptable. **Table 24** shows the *in silico* BET (and BAT) model BMF_L predictions for representative aquatic and terrestrial food webs. The BMFs for fish in the field are higher than the BMFs in the laboratory because the organisms in the field are co-exposed to the chemical in the water as well as their diet.

Table 23. In silico BMF_L model predictions for laboratory fish and rats – TBECH

Metric	Value	Units	Model
In silico lab fish BMF 1	0.13	kg-lw/kg-lw	BET ver. 0.9
In silico lab rat BMF 1	0.092	kg-lw/kg-lw	BET ver. 0.9

Note: Model access date July 2023

Table 24. In silico field BMF_L model predictions – TBECH

Metric	Organism	Value	Units	Model
In silico field BMF 1	Zooplankton	0.51	kg-diet/kg-org [lw]	BET ver. 0.9
In silico field BMF 2	Benthic invertebrate	0.73	kg-diet/kg-org [lw]	BET ver. 0.9
In silico field BMF 3	Aquatic invertebrate	0.55	kg-diet/kg-org [lw]	BET ver. 0.9
In silico field BMF 4	Planktivorous fish	0.50	kg-diet/kg-org [lw]	BET ver. 0.9
In silico field BMF 5	Benthic fish	0.54	kg-diet/kg-org [lw]	BET ver. 0.9
In silico field BMF 6	Omnivorous fish	0.62	kg-diet/kg-org [lw]	BET ver. 0.9
In silico field BMF 7	Piscivorous fish	0.80	kg-diet/kg-org [lw]	BET ver. 0.9
In silico field BMF 8	Aquatic mammal	0.23	kg-diet/kg-org [lw]	BET ver. 0.9
In silico field BMF 9	Small rodent	0.18	kg-diet/kg-org [lw]	BET ver. 0.9
In silico field BMF 10	Terrestrial herbivore	0.33	kg-diet/kg-org [lw]	BET ver. 0.9
In silico field BMF 11	Terrestrial carnivore	0.23	kg-diet/kg-org [lw]	BET ver. 0.9
In silico field BMF 12	Avian passerine	0.35	kg-diet/kg-org [lw]	BET ver. 0.9
In silico field BMF 13	Avian piscivorous	0.45	kg-diet/kg-org [lw]	BET ver. 0.9
In silico field BMF 14	Avian terrestrial	0.34	kg-diet/kg-org [lw]	BET ver. 0.9

Note: Model access date July 2023

Box 6. Evaluation

Problem Statement 1*: Six of the seven in silico BCF model predictions indicate that TBECH is not likely to bioaccumulate in aquatic species. The in silico fish BCF prediction from BET uses the EAS-E Suite consensus biotransformation estimate of 204 h, whereas the Arnot and Gobas BCF models in EPI Suite BCFBAF utilized a shorter half-life of 40 h. In silico fish BAF predictions are more variable, ranging from 676 – 17,000. The BAFs are generally larger than the lab BCFs because the BAF is a field metric and includes dietary exposure that occurs in the environment. The BET BAF predictions are greater than the Arnot and Gobas BAF models in EPI Suite BCFBAF because BCFBAF uses a shorter HL_B than the value used in the BET calculations. Of the 7 fish BAF values, 4 exceed the vB threshold. For invertebrates, all three BAF predictions exceed the vB threshold. Based on divergent outcomes like these from the available LoE one would probably proceed to the next phase of the workflow to address uncertainty in problem statement 1.*

Problem Statement 2*: The in silico lab and food web BMF model predictions indicate that TBECH does not biomagnify in aquatic organisms, i.e., BMF <1. Based on this initial evaluation, one could make the decision to de-prioritize TBECH for biomagnification in aquatic systems. In silico lab and food web BMF model predictions indicate that TBECH does not biomagnify in terrestrial organisms.*

7.2.2.4 Laboratory in vivo bioaccumulation data - TBECH

No OECD 305 TG studies are publicly available for TBECH. However, Gemmill et al., (Gemmill 2011) performed a study with juvenile brown trout (*Salmo trutta*) and calculated BMF_{ss} (wet weight) for TBECH **(Table 25).** Calculated BMFs ranged from 0.17 – 0.32 for three treatment groups. This study was evaluated using a DET **(Table 7)** to generate a reliability score. Details related to the study evaluation and reliability score are included in **Table A C.1.**

Table 25. Summary of fish in vivo BMF data for TBECH

Line of Evidence (LOE)	Organism	SS/K,L	Value	Reliability score	Reference
Lab fish BMF 1	Brown trout	SS	0.066	4.53	(Gemmill 2011)
Lab fish BMF 2	Brown trout	SS	0.017		
Lab fish BMF 3	Brown trout	SS	0.32		

Box 7. Evaluation

Problem Statement 1*: BMF data are not relevant for this problem statement.*

Problem Statement 2: *There are three lab BMFs in the literature for TBECH in fish with a high reliability score. All three values are < 1. These data provide evidence that TBECH does not biomagnify in fish.*

7.2.3 Data Evaluation and Weighing – TBECH

The next phase of the B IATA includes data evaluation and data weighing. Data evaluation uses the DETs described in Section 5.0 to critically evaluate each LoE for reliability. The data weighing step assigns a weight to each LoE by combining the relevance score that was assigned *a priori* within the problem formulation phase with the results from the data evaluation phase.

7.2.3.1 Data Evaluation - TBECH

TBECH is considered a relatively data poor chemical requiring that most of the bioaccumulation data be generated using *in silico* approaches (i.e., inputs to models and outputs from models), with the exception of an *in vitro* biotransformation metric in mammals (Nguyen 2017) and a laboratory fish BMF study (Gemmill 2011). TBECH is a neutral organic structure and as such is amenable to *in silico* predictions well within both the model parameter domain (physical-chemical property) and structural domains of all models used in **Tables 21-25**. Consequently, although empirical evidence is very limited, *in silico* the model results were considered of high quality.

7.2.3.2 Evidence (LoE) Weighing - TBECH

Summaries of the lines of evidence used to evaluate TBECH according to each of the problem statements are shown in **Tables 26-27**. The weight given to each LoE is a function of relevance and reliability. Note that only the LoE for which information was available are reported in the tables. Reliability is a measure of how confident an IATA user is with each line of evidence to support or refute the hypothesis statement in

this example for TBECH according to the regulatory context of B/vB criteria of 2000 L/kg-ww and 5000 L/kg-ww (i.e., for making the decision). In this example, a reliability score is proposed for the *in silico* models based on existing knowledge. Future ongoing work will focus on further defining data quality scores for these models. Users may define their own scales. In this example, we use three non-numerical bins of "low", "moderate", and "high" to convey the weight of each LoE.

Table 26. Summary of LoE to support problem statement 1 for TBECH

LoE	"B" cat.	Relevance	Data Reliability	LoE Weight
In silico fish BCF 1	nB	5	5	High
In silico fish BCF 2	vB	5	5	High
In silico fish BCF 3	nB	5	5	High
In silico fish BCF 4	nB	5	5	High
In silico fish BCF 5	nB	5	5	High
In silico fish BCF 6	nB	5	5	High
In silico fish BCF 7	nB	5	5	High
In silico fish BAF 1	nB	5	5	High
In silico fish BAF 2	nB	5	5	High
In silico fish BAF 3	nB	5	5	High
In silico fish BAF 4	vB	5	5	High
In silico fish BAF 5	vB	5	5	High
In silico fish BAF 6	vB	5	5	High
In silico fish BAF 7	vB	5	5	High
In silico invert BAF 1	vB	3	5	Moderate
In silico invert BAF 2	vB	3	5	Moderate
In silico invert BAF 3	vB	3	5	Moderate

Table 27. Summary of LoE to support problem statement 2 for TBECH

LoE	"B" cat.	Relevance	Data Reliability	LoE Weight
In silico fish BMF 1	nB	4	5	High
In silico rat BMF 1	nB	4	5	High
In silico field BMF 1	nB	4	5	High
In silico field BMF 2	nB	4	5	High
In silico field BMF 3	nB	4	5	High
In silico field BMF 4	nB	4	5	High
In silico field BMF 5	nB	4	5	High
In silico field BMF 6	nB	4	5	High
In silico field BMF 7	nB	4	5	High
In silico field BMF 8	nB	4	5	High
In silico field BMF 9	nB	4	5	High
In silico field BMF 10	nB	4	5	High
In silico field BMF 11	nB	4	5	High
In silico field BMF 12	nB	4	5	High
In silico field BMF 13	nB	4	5	High
In silico field BMF 14	nB	4	5	High
In silico field BMF 15	nB	4	5	High
In vivo fish BMF 1	nB	5	4.53	High
In vivo fish BMF 2	nB	5	4.53	High
In vivo fish BMF 3	nB	5	4.53	High

7.2.4 Bioaccumulation Data Integration and Reporting – TBECH

7.1.4.1 Strength of Evidence

A high-level summary of the various available LoE relevant to the problem statements for TBECH and the corresponding nB, B, and vB classifications of each LoE per the "B" and "vB" criteria thresholds of 2000 L/kg-ww and 5000 L/kg-ww is shown in **Table 28.** In this TBECH example, model results pertaining to problem statement 1 (bioaccumulative in the aquatic environment) are quite variable. Six of the seven *in silico* BCF model predictions indicate that TBECH is not likely to bioaccumulate in aquatic species, while 4 of the 7 fish BAF values exceed the vB threshold. In general terms, the BAFs exceed the lab BCFs because the BAF is a field metric and includes dietary exposure that occurs in the environment. Available data are restricted to *in silico* predictions.

In addition to the disparate outcomes, the lack of *in vitro* and *in vivo* data LoE suggest there are insufficient data to make a high confidence decision for problem statement 1. Fish BCF predictions are relatively consistent (~1000) when assigning a B classification for this substance with a single prediction from the BET model considered an outlier. The higher BET prediction can be partially explained by the larger HL_B (slower predicted biotransformation) compared to the BCFBAF HL_B prediction (**Table 21**). The BCF QSAR models are lower than the BET BCF prediction as well, and although HL_B is not explicitly included in these predictions, the results would indicate the consensus HL_B used in the BET B metric calculations may be too long. Further BET model simulations could include a refined HL_B consensus value by averaging the OPERA and BCFBAF HL_B predictions (increasing n for HL_B predictions from 4 to 6). Reliance on *in silico* data alone may or may not be sufficient for a regulatory conclusion related to problem statement 1,

depending on the context. An evaluator may wish to follow the next step in the workflow by obtaining reliable quality *in vitro* biotransformation rate data for fish and use IVIVE models to further examine the uncertainty in existing HL_B-QSAR predictions. Such a refinement could then be considered in further BET model parameterization and simulations. Barring that decision, one may seek to obtain a reliable quality measured BCF value following OECD 305 testing guidance.

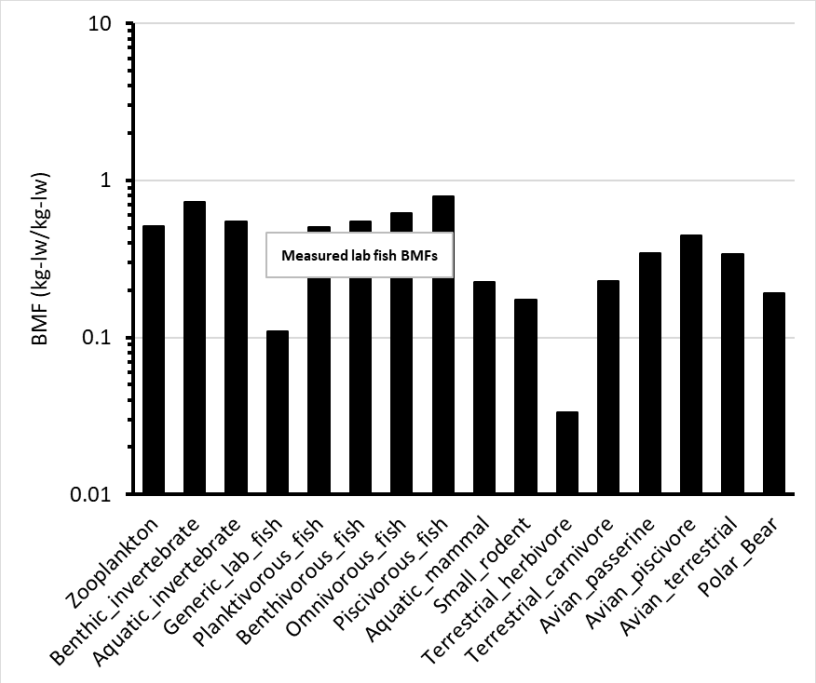
In the case of problem statement 2, all *in silico* predictions and three available high-quality *in vivo* fish BMF studies indicate that TBECH is unlikely to biomagnify in aquatic or terrestrial systems. Depending on the regulatory jurisdiction, these data may be sufficient to supersede the uncertainty in the BCF and BAF categorization.

Table 28. Summary of LoE for TBECH and corresponding "B" classifications per criteria thresholds as specified in each problem statement to indicate strength of evidence

Problem Statement	LoE description	Organism type	# LoE	Total # LoE	%nB	%B	%vB
1	*In silico* BCF	Fish	7	17	53%	0%	47%
	In silico BAF	Fish	7				
	In silico BAF	Invert	3				
2	*In silico* BMF	Fish	1	17	100%	0%	N/A
	In silico BMF	Rat	1				
	In silico field BMF	Various	12				
	In vivo BMF	Fish	3				

Note: Only data considered reliable with relevancy scores > 0 are included within this table.

Figure 10. Summary of existing *in silico* BMF model estimates for TBECH to address problem statement 2



Note: All values are < 1. Black bars represent the in silico predictions, and the white box "measured lab fish BMFs" shows where the highest available empirical in vivo data would fall (i.e., ~0.3 to 0.4 kg-lw/kg-lw) related to the in silico model predictions

7.2.4 Summary – TBECH

TBECH is a relatively "data poor" chemical, and evaluation of problem statement 1 is limited to *in silico* modeling approaches. For problem statement 1, the decision could be made to categorize TBECH as "B" or "vB", the decision could be made to obtain additional model predictions, or the decision could be made to ask for additional data to support or refute the information provided by the current *in silico* predictions, depending on decision context and regulatory relevance. For example, an evaluator may request quality *in vitro* biotransformation rate data for fish and could use IVIVE models to further examine the uncertainty in existing HL_B-QSAR predictions. Such a refinement based on empirical *in vitro* data could then be considered in further BET model parameterization and simulations. Barring that decision, one may seek to obtain a reliable quality measured BCF value following OECD 305 testing guidance. For problem statement 2, the WoE to address problem statement 2 is relatively consistent and coherent, indicating that this chemical is not expected to biomagnify in aquatic or terrestrial systems. **Figure 10** gives a high-level summary of the existing information for TBECH related to problem statement 2.

7.3 Data Rich Example – Phenanthrene (PHE)

7.3.1 Purpose & Hypothesis (Problem Formulation) - PHE

This case example demonstrates the implementation of the B IATA for a relatively "data rich" B assessment scenario. Phenanthrene (PHE; CAS RN 85-01-8) is a naturally occurring polycyclic aromatic hydrocarbon (PAH) in the environment that is also present in crude oil at the start of the petroleum refinement process. Several studies evaluating bioaccumulation and toxicokinetics have been conducted on PHE as well as other related PAHs, and there are considerable data available for this chemical to assess various aspects of bioaccumulation. This example summarizes how to transparently evaluate all data (LoE) consistently and objectively and identify uncertainties and gaps that could be addressed and their impact on decision-making. This can be illustrated using the two following problem statements that will be evaluated with the data:

1. Is there sufficient evidence to support the conclusion that phenanthrene is bioaccumulative in the aquatic environment (referenced to water) based on a "B" criterion of BCF or BAF ≥ 5000 L/kg-ww? [Problem statement 1]
2. Is there sufficient evidence to support the conclusion that phenanthrene biomagnifies in aquatic food webs using the recommended criterion (Burkhard 2013) of 1 kg-lw/kg-lw for neutral hydrophobic organic chemicals? [Problem statement 2]

To address these problem statements, the steps outlined in **Sections 4** (data gathering), **5** (data evaluation) and **6** (data integration and reporting) are followed. To help address the needs of each step, reference is made to the freely available US EPA CompTox Dashboard (https://www.epa.gov/chemical-research/comptox-chemicals-dashboard), the Bioaccumulation Evaluation Tool (BET) implemented in EAS-E Suite (www.eas-e-suite.com), other sources (e.g., EPI Suite (US EPA 2011)), and general B assessment guidance published by Arnot and colleagues (Arnot 2023).

The guidance in the IATA for chemicals that have multiple LoE for multiple B metrics (i.e., relatively data rich) is based on the guidance provided in (Arnot 2023) to aid the WoE approach for B assessment. The BAT (a tool) was also developed to facilitate the implementation of this guidance (Arnot 2023) in a structured scientific method and workflow that is also aligned with the general workflow of this IATA (**Figure 3**). For brevity in this document, many details of data evaluation for PHE are summarily referenced from the original source (Armitage 2021). The critical review by Armitage et al., included the compilation and critical evaluation of 74 measured *in vivo* LoE for fish and invertebrate species from laboratory and field studies. While most of the data used and evaluated in this case example are based on the extensive data generation, compilation, evaluation and integration published in Armitage et al., 2021, there are some minor differences as outlined below.

As stated in **Section 5**, there is no current consensus in the scientific and regulatory communities for relevance weighting for the various B metrics. However, for illustrative purposes, we have assigned the relevance criteria in **Table 29** to the various LoE (0 = least relevant; 5 = most relevant). Although somewhat subjective and variable, it is critical that B metric relevance is communicated in a clear and transparent manner, preferably *a priori* as part of the problem formulation in the assessment process.

For problem statement 1, relevance scores reflect concern for potential bioaccumulation in fish relative to water concentrations resulting from uptake from all exposure sources (i.e., via the gills, diet and dermal contact). Measured studies are given higher overall relevance than *in silico* results. The lower scores are indicative of a lower relevance given to non-fish species (invertebrates) resulting from little or no regulatory

precedence as sentinel bioaccumulation species and scientific uncertainty with bioaccumulation metrics measured in such species (e.g., uptake rates, biotransformation capacity). The relevance of invertebrate data is context dependent and may be more relevant in some jurisdictions.

For problem statement 2, relevance scores reflect concern for potential biomagnification in aquatic food webs. Therefore, LoE that provide specific evidence of biomagnification (e.g., BMF and TMF) are given high relevancy scores, with empirical data given higher scores than *in silico* models. While the BAF does not directly provide biomagnification information (i.e., BMF or TMF), it can be converted to a fugacity ratio (Burkhard et al. 2012, Armitage et al., 2021) that can provide such information. If the fugacity ratio is greater than 1, this indicates the chemical has biomagnified. If measured BMF or TMF data are not available this method helps address the lack of measured biomagnification information in a WoE approach.

Table 29. Relevance scores assigned a priori to each LoE for PHE for each problem statement

Problem stmt.	LoE description	Organism type	Relevance Score (0 = least; 5 = most)	Relevance score description
1	*In silico* lab BCF	Fish	4	Focus on fish; *in silico* lower score than *in vivo*
	In silico BAF	Fish	4	Focus on fish; *in silico* lower score than *in vivo*
	In silico BAF	Invert	2	*In silico* lower score than *in vivo*
	In silico lab BMF	Fish	0	Criteria specific to BCF / BAF
	In silico lab BMF	Rat	0	Focus on fish; mammals not in scope
	In silico field BMF	Various	0	Criteria specific to BCF / BAF
	In vivo lab BCF	Fish	5	Focus on fish; *in vivo* higher score than *in silico*
	In vivo lab BCF	Invert	3	*In vivo* higher score than *in vivo*
	In vivo lab BMF	Fish	0	Not relevant for this specific example; could be in other jurisdictions; higher uncertainty
	In vivo field BAF	Various	4	Focus on fish; *in vivo* higher score than *in silico*
	In vivo field BMF	Various	0	Criteria specific to BCF / BAF
	In vivo field TMF	Various	0	Criteria specific to BCF / BAF
2	*In silico* lab BCF	Fish	0	Not relevant for biomagnification
	In silico BAF	Fish	0	Not relevant for biomagnification
	In silico BAF	Invert	0	Not relevant for biomagnification
	In silico lab BMF	Fish	4	Specific biomagnification metric; *in silico* lower score than *in vivo*
	In silico lab BMF	Rat	0	Focus on aquatic species
	In silico field BMF	Various	4	Specific biomagnification metric; *in silico* lower score than *in vivo*
	In vivo lab BCF	Fish	0	Not relevant for biomagnification
	In vivo lab BCF	Invert	0	Not relevant for biomagnification
	In vivo lab BMF	Fish	5	Specific biomagnification metric; *in vivo* higher score than *in silico*
	In vivo field BAF	Various	2	While the BAF does not directly provide biomagnification information (i.e., BMF or TMF), it can be converted to a fugacity ratio; *in vivo* data would be useful
	In vivo field BMF	Various	5	Specific biomagnification metric; *in vivo* higher score than *in silico*
	In vivo field TMF	Various	5	Specific biomagnification metric; *in vivo* higher score than *in silico*

7.3.2 Approach - PHE

A detailed and extensive evaluation for most of the publicly available PHE data that could be used in a B assessment has been previously published and can be referenced for additional details and information (Armitage 2021). It should be noted that at any stage of the workflow (**Figure 3**), one can proceed to evaluate relevance, reliability and quality, sufficiency, and consistency before determining whether the next phase needs to be completed or if there is sufficient confidence in the WoE to decide for each of the two problem statements. This general workflow highlights the flexibility of the IATA for addressing different objectives (e.g., screening-level vs. definitive conclusion) and subjective differences (professional

judgement, jurisdiction requirements on different B metrics) that may occur when conducting a B assessment.

7.3.2.1 Physical-Chemical Properties - PHE

Following the problem formulation phase, the initial step is to gather relevant physical-chemical property information for PHE, starting with the critical information needed to apply these data against regulatory screening values and parameterize available BCF, BAF and BMF prediction models. The screening criteria are driven by K_{OW} and K_{OA}; however, other rudimentary properties (e.g., vapor pressure, water solubility, Henry's Law constant) are helpful when determining consistency and confidence in the selected K_{OW} and K_{OA} values used in the assessment. Molar mass is relevant for converting between molar and mass-based concentration data. PHE is a neutral chemical, so pKa is not needed. It is important to note that there are currently no evaluation templates to critically evaluate physical-chemical property information; however, it is recognized that measured values for well-studied chemicals can have significant errors and there is merit in evaluating physical-chemical properties for reliability. To address this, Li et al., (Li 2022) provide some guidance for obtaining, evaluating, and selecting suitable physical-chemical property information for chemical evaluations. For properties obtained from *in silico* methods, the generic DET for QSAR based predictions developed by Arnot and colleagues (Arnot 2023) and included the BAT for biotransformation half-lives and BCFs (i.e., see **Table 4**) can be considered to determine possible sources of uncertainty.

A summary of the physical-chemical property information for PHE obtained from various sources via the USEPA CompTox Dashboard and EAS-E Suite is shown in **Table 30.** For the purpose of this case study, experimental property data were chosen over modeled estimates and these measured property values were deemed to be of high quality and reliable for this case study and in the Armitage et al. publication (Armitage 2021). Briefly, if more than one reliable physical-chemical property experimental value was available, the average of the values was used, which is clearly stated in **Table 30**. In the case of PHE, experimental values are available for all the relevant physical-chemical parameters required for screening and model parameterization. However, if modeled values are used, it is necessary to ensure that the chemical is within the domain of the model prediction. For illustrative purposes, the modeled values that were within the applicability domain of the specified models (according to EAS-E Suite and/or the USEPA CompTox Dashboard) are included in **Table 30**. By arranging the property information and clearly noting which value(s) were used in the assessment, transparency is ensured and a rationale for not using a particular value can be provided.

Table 30. Experimental & estimated physical-chemical properties for PHE

Property (value used)	Value	Units	Source
MW (178.08)	178.08	g/mol	
log Kow (4.47)	4.47 (exp)	Unitless	(Hansch 1995)
	4.46 (exp)		PhysPropNCCT
	4.47 (est)		OPERA 2.6
	4.68 (est)		ACD/Labs
	4.71 (est)		ACD/Labs Consensus
	4.37 (est)		KOWWIN_v1.69-AFC/IFSQSAR LogKOW-ppLFER_v1.0
	4.35 (est)		KOWWIN_v1.69-AFC
	4.38 (est)		IFSQSAR LogKOW-ppLFER_v1.0
	4.52 (est)		OPERA logP-KNN
Log Koa (7.57)	7.57 (exp)	Unitless	Harner, 1998
	7.49 (est)		KOAWIN_v1.11-Exp-based calculation/IFSQSAR LogKOA-ppLFER_v1.0
	7.22 (est)		KOAWIN_v1.11-Exp-based calculation
	7.76 (est)		IFSQSAR LogKOA-ppLFER_v1.0
	7.03 (est)		KOAWIN_v1.11-KowWin/HenryWin
	7.50 (est)		OPERA logKOA-KNN
	7.55 (est)		OPERA 2.6
VP (1.61e-2)	1.61e-2 (exp)	Pa	(Sonnefeld 1983)
	5.76e-3 (est)		MPBPVPWIN_v1.44-Modified Grain Method
	6.53e-3 (est)		TEST
	1.49e-2 (est)		OPERA 2.6
	2.75e-2 (est)		ACD/Labs
WS (6.33e-6) *Avg of 6 exp values*	6.31e-6 (exp)	mol/L	Schwarz, 1977
	4.52e-6 (est)		WATERNT_v1.02-AFC/WSKOWWIN_v1.43-logKOW regression
	5.38e-6 (est)		WATERNT_v1.02-AFC
	3.80e-6 (est)		WSKOWWIN_v1.43-logKOW regression
	5.50e-6 (exp)		(Tetko 2001)
	6.17e-6 (exp)		(Ran 2001)
	6.45e-6 (exp)		PhysPropNCCT
	6.46e-6 (exp)		(Kovdienko 2010)
	7.08e-6 (exp)		(Hughes 2008)
	2.50e-6 (est)		ACD/Labs
	1.45e-6 (est)		OPERA 2.6
	3.55 e-6 (est)		TEST

Note: Values selected to utilize for this assessment are noted in parentheses in the first column. Model access date July 2023.

Box 8. Initial evaluation

Based on this initial collection of physical-chemical properties, the log K_{OW} is < 5. The log K_{OW} of PHE is > 2 and the log K_{OA} is > 5 indicating the potential for bioaccumulation in air-breathing organisms, and therefore additional evaluation is needed (not included in this case example, see case examples 1 and 2 for evaluation of B potential in air-breathing organisms). Of course, moving to the next step of the IATA will depend on the problem formulation and specific regulatory context. For example, some regulations may require a full systematic review for registration purposes[1], whereas for screening assessments, it may be sufficient to stop the evaluation based solely on physical-chemical property

information. Even though this chemical does not surpass the screening level criteria and a decision could be made at this stage, for illustrative purposes, we determine that there is not sufficient confidence to answer either of the problem statements (1 & 2) and therefore proceed to the next step of the workflow.

Source: [1] (US EPA 2021)

7.3.2.2 Biotransformation - PHE

It is well-documented that an organism's capacity to biotransform a chemical can have a significant impact on bioaccumulation potential for hydrophobic organic chemicals (Nichols 2007; Arnot 2008b; McLachlan 2011). Estimates of whole-body biotransformation rates can be obtained via *in silico, in vitro, and in vivo* models, and these values can then be utilized within existing bioaccumulation models. For PHE, whole-body level biotransformation half-lives (HL_B) are available or can be estimated for fish and are summarized in **Table 31.** This biotransformation rate information can be used to parameterize toxicokinetic BCF, BAF and BMF models. Bioaccumulation models in the BET module in the EAS-E Suite platform can be applied. One can select which biotransformation parameters should be used based on an evaluation of the model applicability domains (e.g., QSAR models) or data quality evaluations (e.g., *in vivo* or *in vitro* studies), or professional judgement. As described in **Section 5.1**, DETs are available to evaluate each biotransformation estimate and determine its suitability for use. *In vivo* studies reviewed in Arnot 2008 (Arnot 2008c) were subject to an extensive data evaluation process which is described therein. The DETs for the *in vitro* studies listed in **Table 31** are provided in Armitage et al., 2021. Lastly, the IFSQSAR and QSARINS predictions all have QSAR Model Reporting Formats (QMRFs) that can be accessed and reviewed during the evaluation process.

Table 31. Summary of whole-body biotransformation half-life data normalized to 0.01 kg body mass fish ($HL_{B,N}$) data for PHE

Metric	Species	Value (h)	Source / notes
Half-life (whole-body) *In silico prediction*	Fish (@ 0.01 kg)	96.2	OPERA 2.6
		61.3	EPI Suite 4.11-Fish Biotrans. HL-BCFBAFv3.01
		33.3	IFSQSAR fhlb-IFS_v1.0
		43.5	QSARINSChem logHLn M1
		46.2	QSARINSChem logHLn M2
		45.7	QSARINSChem. logHLn M3
Half-life (whole-body) *In vivo*	Fish - Turbot	37.3	(Arnot 2008b)
	Fish – Sheepshead minnow	40.6	
	Fish – Sheepshead minnow	44.5	
	Fish – Rainbow trout	108.1	
Half-life (whole-body) *In vitro*	Fish – Rainbow trout (S9)	61.4*	(Nichols 2018b)
		78.2*	(Nichols 2019)

**In vitro* data scaled to $HL_{B,N}$ using IVIVE models.
Note: Model access date July 2023

Box 9. Evaluation

For fish, the results from 6 in silico $HL_{B,N}$ predictions, 4 available "good" or "moderate" quality in vivo studies, and two in vitro S9 studies show strong consistency (range = 33.3 – 108.1). For the purpose of this evaluation, an $HL_{B,N}$ for fish of 50 h was chosen based on the approach to weighting the various data streams as described in Armitage et al. 2021. This value can be used with high confidence to parameterize the BET in silico BCF, BAF, and BMF models.

7.3.2.3 In silico B Predictions - PHE

The next step after obtaining physical-chemical property and biotransformation rate information is to utilize existing models to generate modeled bioaccumulation predictions. This is important even in the case of a very data-rich chemical like PHE, because gathering and critically evaluating existing studies is very time-consuming. In some cases, having some initial screening level modeling information can aid in more strategic and targeted data collection efforts. Furthermore, in some cases the model predicted values can highlight inconsistencies or errors in the empirical data.

A summary of the *in silico* BCF and BAF predictions for PHE is shown in **Table 32.** This table includes a few more *in silico* predictions than were originally presented in Armitage et al., 2021. The propagation of uncertainty in the $HL_{B,N}$ values in the BAT model calculations is described within the Armitage et al. 2021 publication. Based on available information, all these predictions are within the applicability domain of the models and are of high quality (reliability). Some differences in BCF predictions occur because of differences in the lipid content of the modelled organisms and the modeling approach. BAFs are fundamentally different than BCFs because they include dietary exposures as occurs in the environment,

while BCFs are laboratory endpoints based on water exposures only. Thus, for the same chemical, BAFs are typically higher than BCFs (e.g., Arnot and Gobas, 2006). However, differences in trophic position, lipid contents, and biotransformation rates result in variability in BAFs. The data show general consistency with respect to the B categorization.

Table 32. In silico lab BCF and field BAF model predictions for aquatic organisms – PHE

Metric	Value	Units	Model
In silico fish BCF 1	2510	L/kg-ww	OPERA 2.6
In silico fish BCF 2	912	L/kg-ww	BET ver. 0.9 (total water concentration)
In silico fish BCF 3	942	L/kg-ww	BET ver. 0.9 (dissolved water concentration)
In silico fish BCF 4	1860	L/kg-ww	BCFBAF - EPI Suite (regression-based estimate)
In silico fish BCF 5	1203	L/kg-ww	BCFBAF – EPI Suite (Arnot & Gobas; upper TL)
In silico fish BCF 6	1145	L/kg-ww	BCFBAF – EPI Suite (Arnot & Gobas; mid TL)
In silico fish BCF 7	1096	L/kg-ww	BCFBAF – EPI Suite (Arnot & Gobas; lower TL)
In silico fish BCF 8	634	L/kg-ww	TEST
In silico fish BAF 1	1242	L/kg-ww	BCFBAF – EPI Suite (Arnot & Gobas; upper TL)
In silico fish BAF 2	1248	L/kg-ww	BCFBAF – EPI Suite (Arnot & Gobas; mid TL)
In silico fish BAF 3	1289	L/kg-ww	BCFBAF – EPI Suite (Arnot & Gobas; lower TL)
In silico zooplankton BAF	935	L/kg-ww	BET ver. 0.9 (total water concentration)
In silico benthic invertebrate BAF	967	L/kg-ww	BET ver. 0.9 (total water concentration)
In silico aquatic invertebrate BAF	1060	L/kg-ww	BET ver. 0.9 (total water concentration)
In silico planktivorous fish BAF	1200	L/kg-ww	BET ver. 0.9 (total water concentration)
In silico benthic fish BAF	1280	L/kg-ww	BET ver. 0.9 (total water concentration)
In silico omnivorous fish BAF	1610	L/kg-ww	BET ver. 0.9 (total water concentration)
In silico piscivorous fish BAF	2340	L/kg-ww	BET ver. 0.9 (total water concentration)

Note: Model access date July 2023

Table 33 shows the *in silico* BET (and BAT) model BMF_L prediction for a representative lab fish. The model simulates an OECD 305 bioaccumulation test for dietary exposures and the subsequent BMF_L metric in a typical 0.01 kg, 5% lipid content fish. The input parameters (K_{OW} and $HL_{B,N}$) used in this simulation are considered to be of high quality (reliability) and the ensuing BMF prediction is also considered to be of high quality.

Table 33. In silico BMF_L model predictions for a representative laboratory fish – PHE

Metric	Value	Units	Model
In silico fish lab BMF 1	0.021	kg-lw/kg-lw	BET ver. 0.9

Note: Model access date July 2023

The *in silico* BET (and BAT) model BMF_L predictions for various aquatic organisms in a representative aquatic food web (Arnot 2023) are shown in **Table 34.** Differences in trophic position, lipid contents, and biotransformation rates as well as the relative flux of chemical into the organism from water and diet result in variability in BMFs. The input parameters (K_{OW} and $HL_{B,N}$) used in this simulation are considered high quality (reliability) and the ensuing BMF_L prediction are also considered high quality. The field BMFs are higher than the lab simulated BMFs because organisms in the environment are also simultaneously exposed to chemical in the water, whereas in the laboratory OECD 305 model simulation the fish is only exposed to chemical in its diet (no co-exposure to the chemical in water).

Table 34. In silico food web BMF_L model predictions – PHE

Metric	Organism	Value	Units	Model
In silico field BMF 1	Zooplankton	0.24	kg-diet/kg-org [lw]	BET ver. 0.9
In silico field BMF 3	Aquatic invertebrate	0.52	kg-diet/kg-org [lw]	BET ver. 0.9
In silico field BMF 4	Planktivorous fish	0.53	kg-diet/kg-org [lw]	BET ver. 0.9
In silico field BMF 5	Benthic fish	0.59	kg-diet/kg-org [lw]	BET ver. 0.9
In silico field BMF 6	Omnivorous fish	0.68	kg-diet/kg-org [lw]	BET ver. 0.9
In silico field BMF 7	Piscivorous fish	0.85	kg-diet/kg-org [lw]	BET ver. 0.9

Note: Model access date July 2023

Box 10. Evaluation

Problem Statement 1: *For aquatic species, all the in silico BCF model predictions predict BCFs < 5,000 L/kg-ww. All in silico BAF predictions are also < 5,000 L/kg-ww. The decision could be made at this stage to utilize the concordance of the various in silico model estimates for aquatic BCF and BAF and conclude that PHE is nB in aquatic systems based on the criteria applied in problem statement 1. For the purpose of this case study, we will continue to the next phase of the evaluation to include measured bioaccumulation data.*

Problem Statement 2: *The in silico lab and field BMF model predictions indicate that PHE does not biomagnify in aquatic organisms, i.e., BMF < 1 kg-lw/kg-lw. Based on this initial evaluation, one could make the decision to de-prioritize PHE for biomagnification in aquatic systems based on the criteria applied in problem statement 2. However, for the purpose of this case study, we will proceed to the next phase of the evaluation to include measured biomagnification data.*

7.3.2.4 Laboratory in vivo bioaccumulation data - PHE

All laboratory BCF and BMF data for aquatic species used in this example were critically evaluated and provided in the Supplemental Data sections of Armitage et al., (Armitage 2021). As detailed in Armitage et al., 2021, there are inconsistencies in the reporting of units in the available *in vivo* studies (e.g., wet, dry, and lipid-basis) and lack of ancillary data on organism composition (e.g., lipid and water content) which makes it difficult to evaluate and standardize the invertebrate BCF data. In Armitage et al., 2021 all invertebrate BCF data were converted to wet weight values with an assumed whole-body lipid content of 5%. However, as discussed in (Armitage 2021), the method for standardizing BCF values to a 5% lipid content is not appropriate for chemicals subject to appreciable biotransformation like PHE; the

standardization method is only appropriate for very bio-persistent chemicals. Both approaches were used in the Armitage et al. publication. For the focus of this case example, we preferentially selected the more appropriate (non-standardized) wet weight BCFs. Furthermore, for the purposes of this illustrative example, studies with reliability scores of 0 (critical fail) from Armitage et al., (Armitage 2021) are not shown in the summary tables below. For transparency, the list of published B studies for PHE not included in this case study due to significant study limitations and/or critical data quality issues (e.g., reliability scores of 0) are provided in **Annex D**. One example of a significant error in reported BCFs is identified in the Carlson et al. (1979) study. According to OECD 305 BCF TG (2012), a requirement for valid BCFs is that the fish tissue and water concentrations are maintained near constant values (± 20% of the mean) during the exposure period in which the steady-state BCF ratio is calculated and ideally this covers 3 sampling periods. The Carlson et al., 1979 study results show that this requirement was not satisfied in their experiments (i.e., experiment #2, Tank #2 fish concentrations were increasing over time and water concentrations were decreasing over time); therefore, BCFs like this are not considered reliable. Moreover, in this "data rich" case study there are several reliable quality BCF studies that can be included in the WoE approach. For detailed information on the calculation of reliability scores, including identified study limitations, for PHE please see (Armitage 2021).

7.3.2.4.1 *In vivo BCF Data (fish) – PHE*

A total of 17 *in vivo* lab BCF tests for PHE were critically reviewed in Armitage et al., (Armitage 2021). **Table 35** provides a summary of the reliability scoring and BCF values for fish for those with reliability scores > 0. Excluding a possible outlier, lab fish BCF 11, the BCFs range about a factor of 5 from the lowest reliable BCF to the highest. This is in general agreement with the natural variability that exists for BCF measurements (Arnot 2006).

Table 35. Summary of fish in vivo BCF Data for PHE

Line of Evidence (LOE)	Organism	SS/K	Value	Reliability score	Reference
Lab Fish BCF 1	Turbot-*Scophthalmus maximus*	SS	309	2.41	(Baussant 2001)
Lab Fish BCF 2	Turbot-*Scophthalmus maximus*	K	936	3.07	(Baussant 2001)
Lab Fish BCF 3	Golden ide-*Leuciscus idus melanotus*	SS	1760	2.13	(Freitag 1985)
Lab Fish BCF 4	Sheepshead minnow	SS	1623	3.59	(Jonsson 2004)
Lab Fish BCF 5	Sheepshead minnow	SS	700	3.59	(Jonsson 2004)
Lab Fish BCF 6	Zebrafish-*Danio rerio*	K	900	4.35	(Li 2018)
Lab Fish BCF 7	Rainbow trout-*Oncorhynchus mykiss*	K	690	3.26	(Lo 2016)
Lab Fish BCF 8	Zebrafish-*Danio rerio*	K	584	4.09	(Wang 2018)
Lab Fish BCF 9	*Pseudopleuronectes yokohamae*	K	1040	3.80	(Kobayashi 2013)
Lab Fish BCF 10	Zebrafish-*Danio rerio*	K	585	4.46	(Xia 2015)
Lab Fish BCF 11	Zebrafish-*Danio rerio*	SS	52	4.24	(Wang 2019)

Box 11. Evaluation

Problem Statement 1: *There are 11 laboratory fish BCF studies in six different species with acceptable reliability scores for PHE. All studies had BCF values < 5,000 L/kg-ww. The BCFs ranged more than 30-fold (from 52 to 1760) across all species and as much as 15-fold (52 to 900) within a single species (zebrafish). The geometric mean from the 11 reliable quality lab BCFs is 634 L/kg-ww and the 95th percentile confidence intervals of the geometric mean are 332 to 1209. The calculation of a geometric mean is exclusively intended to convey central tendency and data variation; however, these statistical values were not explicitly used in the WoE approach. Based on the consistency of the results, one could conclude that PHE is nB in aquatic systems based on the criteria applied in problem statement 1; however, for the purpose of this case study, we will move forward to the next phase of the evaluation.*

Problem Statement 2: *The BCF data are not relevant for evaluating biomagnification.*

7.3.2.4.2 *In vivo BCF Data (invertebrate) - PHE*

Though the experimental invertebrate data summarized in **Table 36** were not generated following an OECD Test Guideline, in the interest of using all available data, we have included them in this case study. It is important to note that because there are no standardized guidelines for these tests, reliability scoring is based on the same *in vivo* BCF DET that was developed for fish studies (Arnot 2023) which is based on the OECD 305 Testing Guidance for fish (OECD 2012). Some discussion of issues related to use and evaluation of invertebrate studies for the B assessment of PHE is included in Armitage et al. (Armitage 2021). **Table 36** provides a summary of the reliability scoring and BCF values for invertebrates. It is noted that the variability in BCFs for invertebrates is relatively large (about a factor of 20) and there are no clear outliers.

Table 36. Summary of invertebrate in vivo BCF Data for PHE

Line of Evidence (LOE)	Organism	SS/K	Value	Reliability score	Reference
Lab Invert BCF 1	*Mytilus edulis*	SS	2932	3.44	(Baussant 2001)
Lab Invert BCF 2	*Stylodrilus heringlanus*	K	1040	2.67	(Frank 1986)
Lab Invert BCF 3	*Calanus finmarchicus*	SS	265	2.89	(Jensen 2012)
Lab Invert BCF 4	*Calanus hyperboreus*	SS	2970	3.2	(Agersted 2018)
Lab Invert BCF 5	*Daphnia pulex*	SS	374	2.54	(Southworth 1978)
Lab Invert BCF 6	*Daphnia magna*	SS	161	4.24	(Wang 2019)

Box 12. Evaluation

Problem Statement 1: *There are 6 laboratory invertebrate BCF studies in six different species with acceptable reliability scores for PHE, all with BCF values < 5,000 L/kg-ww. The BCFs ranged more than 18-fold (from 161 to 2970) across all species. The geometric mean from the 6 reliable quality lab BCFs is 724 L/kg-ww and the 95th percentile confidence intervals of the geometric mean are 196 to 2684. The calculation of a geometric mean is exclusively intended to convey the central tendency and data variation; however, these statistical values were not explicitly used in the WoE approach. Based on the consistency of the results, one could conclude that PHE is nB in aquatic systems based on the criteria applied in problem statement 1; however, for the purpose of this case study, we will move forward to the next phase of the evaluation.*

Problem Statement 2: *The BCF data are not relevant for evaluating biomagnification.*

7.3.2.4.3 *In vivo lab BMF data (fish) – PHE*

A summary of two *in vivo* laboratory BMFs for fish that are available for PHE are shown in **Table 37**. The reliability scores are based on the DET developed for laboratory BMF studies (Arnot 2023) which is based on the OECD 305 Testing Guidance (OECD 2012). There is general consistency between the two reliable measured *in vivo* values. It is recognized, however, that lab BMF data are not the same as field BMF data in which the latter are co-exposed to the water simultaneously.

Table 37. Summary of fish in vivo lab BMF data for PHE.

Line of Evidence (LOE)	Organism	SS/K,L	Value	Reliability score	Reference
Lab fish BMF 1	Rainbow trout-*Oncorhynchus mykiss*	K, L	0.066	4.38	(Gobas 2019b)
Lab fish BMF 2	Zebrafish-*Danio rerio*	K, L	0.017	4.25	(Wang 2019)

Box 13. Evaluation

Problem Statement 1: *BMF data are not relevant for this problem statement.*

Problem Statement 2: *There are two reliable measured lipid normalized lab BMFs for PHE in fish. Both values are < 1. These data provide evidence that PHE does not biomagnify in fish under laboratory exposures. For the purposes of this case study, we will continue to the next phase of the evaluation.*

7.3.2.5 Field-based in vivo bioaccumulation data – PHE

7.3.2.5.1 Field BAF data – PHE

A total of 8 field BAFs for PHE were collected and evaluated for data quality using the field BAF DETs (Armitage 2021). As outlined in Armitage et al., (Armitage 2021) many of these BAFs were determined to be unreliable (critical fails) for B assessment for various reasons. Two field BAFs were reliable, and those values and their scores are summarized in **Table 38**.

Table 38. Summary of reliable fish field BAF data for PHE

Line of Evidence (LOE)	Organism	SS/K,L/5%	Value	Reliability score	Reference
Field BAF 1	*Acanthogobius flavimanus*	SS, 5%	418	3.04	(Takeuchi 2009)
Field BAF 2	Crucian carp	SS	619	3.75	(Ke 2007)

Box 14. Evaluation

Problem Statement 1: *There are 2 reliable field BAFs for PHE in fish. They are both < 5,000 L/kg-ww indicating the PHE is nB in aquatic organisms based on the criteria applied in problem statement 1. For the purposes of this case study, we will continue to the next phase of the evaluation.*

Problem Statement 2: *The BAF data could be used in a WoE approach for evaluating biomagnification by converting them to fugacity ratios (Burkhard et al., 2012; Armitage et al., 2021). However, in this case example we do not perform this analysis.*

7.3.2.5.2 Field BMF data

A summary of the available Field BMF studies as outlined in Armitage et al. (Armitage 2021) is shown in **Table 39**. In this case example, studies that were identified as having critical fails per the DETs are not included.

Table 39. Summary of reliable fish field BMF data for PHE

Line of Evidence (LoE)	Organism	SS/K,L/5%	Value	Reliability score	Reference
Field BMF 1	Herring	SS,L	0.29	1.52	(Nfon 2008)
Field BMF 2	"fish"	SS,L	0.0068	2.41	(Moermond 2007)

Box 15. Evaluation

Problem Statement 1: *BMF data are not relevant for this problem statement.*

Problem Statement 2: *There are two measured lipid normalized lab BMFs for PHE in fish. Both values are < 1. These data provide clear evidence that PHE does not biomagnify in fish based on the criteria applied in problem statement 2. For the purposes of this case study, we will continue to the next phase of the evaluation.*

7.3.2.5.3 Field TMF data

A summary of the available Field TMF studies as outlined in Armitage et al. (Armitage 2021) is shown in **Table 40.** In this case example, studies that were identified as having critical fails per the DETs are not included.

Table 40. Summary of reliable fish field BMF data for PHE

Line of Evidence (LoE)	Value	Reliability score	Reference
Field TMF 1	0.34	3.84	(Khairy 2014)
Field TMF 2	0.74	2.88	(Nfon 2008)

Box 16. Evaluation

Problem Statement 1: *TMF data are not relevant for this problem statement.*

Problem Statement 2: *There are two reliable measured TMFs for PHE in aquatic food webs. Both values are < 1 at a statistical significance of < 0.05 (see Armitage et al. for further details). These data provide clear evidence that PHE does not biomagnify in fish based on the criteria applied in problem statement 2.*

7.3.3 Data Evaluation and Weighing – PHE

The next phase of the B IATA includes data evaluation and data weighing. Data evaluation uses the DETs described in Section 5.0 to critically evaluate each LoE for reliability. The data weighing step assigns a weight to each LoE by combining the relevance score that was assigned *a priori* within the problem formulation phase with the results from the data evaluation phase.

7.3.3.1 Data Evaluation - PHE

A summary of each LoE for PHE is provided in **Tables 41-42.** The relevance scores were assigned at the start of the assessment and are based on the evaluator's needs and regulatory context. Reliability of each LoE is based on the reliability scores calculated from the available DETs within the BAT. In the summary section (worksheet) of the BAT there is a summary of the detailed information related to the reliability of each LoE such that it can be transparently documented and communicated. Weight given is entirely a user-defined choice, based on the combination of information within the relevance and reliability columns.

Table 41. Summary of LoE to support problem statement 1 for PHE, with the "B" categorization defined as BCF or BAF ≥ 5000 L/kg-ww

LoE	"B" cat.	Relevance	Data Reliability	LoE Weight
In silico fish BCF 1	nB	4	5	High
In silico fish BCF 2	nB	4	5	High
In silico fish BCF 3	nB	4	5	High
In silico fish BCF 4	nB	4	5	High
In silico fish BCF 5	nB	4	5	High
In silico fish BCF 6	nB	4	5	High
In silico fish BCF 7	nB	4	5	High
In silico fish BCF 8	nB	4	5	High
In silico fish BAF 1	nB	4	5	High
In silico fish BAF 2	nB	4	5	High
In silico fish BAF 3	nB	4	5	High
In silico fish BAF 4	nB	4	5	High
In silico fish BAF 5	nB	4	5	High
In silico fish BAF 6	nB	4	5	High
In silico fish BAF 7	nB	4	5	High
In silico invert BAF 1	nB	2	5	Moderate
In silico invert BAF 2	nB	2	5	Moderate
In silico invert BAF 3	nB	2	5	Moderate
Lab Fish BCF 1	nB	5	2.41	Moderate
Lab Fish BCF 2	nB	5	3.07	High
Lab Fish BCF 3	nB	5	2.13	Moderate
Lab Fish BCF 4	nB	5	3.59	High
Lab Fish BCF 5	nB	5	3.59	High
Lab Fish BCF 6	nB	5	4.35	High
Lab Fish BCF 7	nB	5	3.26	High
Lab Fish BCF 8	nB	5	4.09	High
Lab Fish BCF 9	nB	5	3.80	High
Lab Fish BCF 10	nB	5	4.46	High
Lab Fish BCF 11	nB	5	4.24	High
Lab Invert BCF 1	nB	3	3.44	Moderate
Lab Invert BCF 2	nB	3	2.67	Low
Lab Invert BCF 3	nB	3	2.89	Low
Lab Invert BCF 4	nB	3	3.2	Moderate
Lab Invert BCF 5	nB	3	2.54	Low
Lab Invert BCF 6	nB	3	4.24	Moderate

Table 42. Summary of LoE to support problem statement 2 for PHE

LoE	"B" cat.	Relevance	Data Reliability	LoE Weight
In silico fish lab BMF 1	nB	4	5	High
In silico field BMF 1	nB	4	5	High
In silico field BMF 2	nB	4	5	High
In silico field BMF 3	nB	4	5	High
In silico field BMF 4	nB	4	5	High
In silico field BMF 5	nB	4	5	High
In silico field BMF 6	nB	4	5	High
In silico field BMF 7	nB	4	5	High
In vivo lab BMF 1	nB	5	4.38	High
In vivo lab BMF 2	nB	5	4.25	High
In vivo field BAF 1	nB	2	3.04	Low
In vivo field BAF 2	nB	2	3.75	Low
In vivo field BMF 1	nB	5	1.52	Moderate
In vivo field BMF 2	nB	5	2.41	Moderate
In vivo field TMF 1	nB	5	3.84	High
In vivo field TMF 2	nB	5	2.88	Moderate

7.2.4 Bioaccumulation Data Integration and Reporting – PHE

7.3.4.1 Strength of Evidence - PHE

A high-level summary of the various available LoE for PHE and the corresponding nB and B classifications of each LoE per the selected "B" assessment criteria outlined in the problem formulation stage is shown in **Table 43.** The bioaccumulation data are consistent and coherent between *in silico* predictions and laboratory and field data.

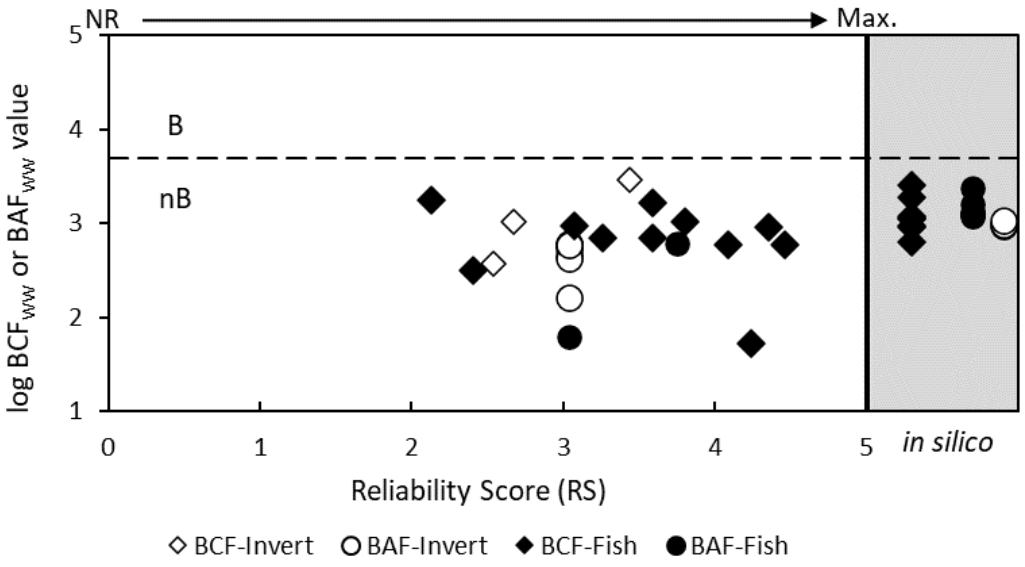
Table 43. Summary of LoE for PHE and corresponding "B" classifications per criteria thresholds as specified in each problem statement to indicate strength of evidence

Problem Statement	LoE description	Organism type	# LoE	Total # LoE	%nB	%B
1	*In silico* lab BCF	Fish	8	35	100%	0%
	In silico lab BAF	Fish	7			
	In silico lab BAF	Invert	3			
	Lab *in vivo* BCF	Fish	11			
	Lab *in vivo* BCF	Invert	6			
2	*In silico* lab BMF	Fish	1	14	100%	0%
	In silico foodweb BMF	Various	7			
	Lab BMF	Fish	2			
	Field BMF	Fish	2			
	Field TMF	Various	2			

Note: Only data considered reliable with relevancy scores >0 are included within this table.

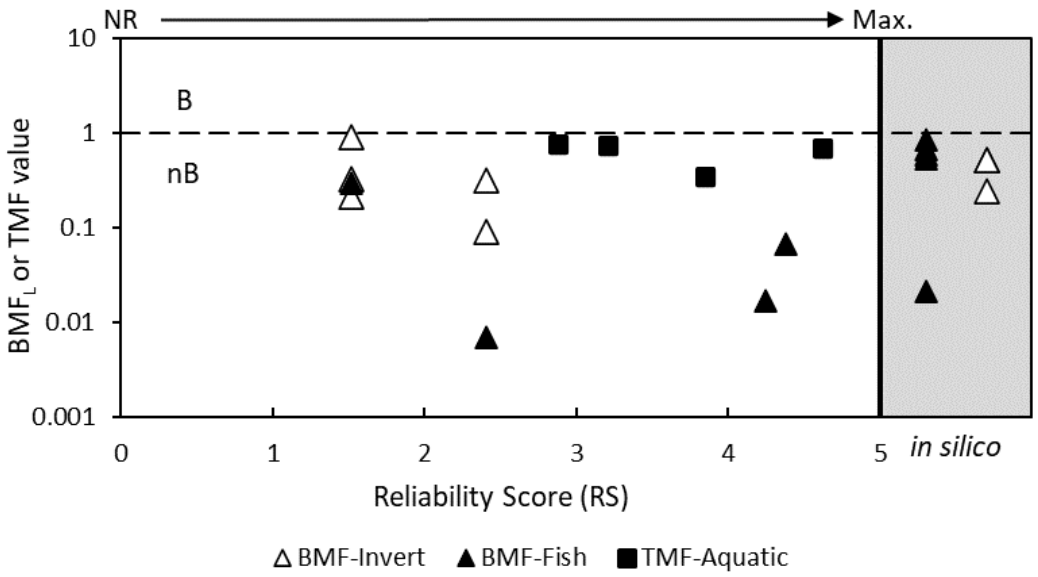
A summary of the reliability scores for the BCF and BAF values for fish and invertebrates in comparison to the "B" criterion threshold of 5000 L/kg-ww is provided in **Figure 11.** The reliability scores reflect the relative quality of the various LoE based on data evaluation methods derived from OECD Testing Guidance and expert judgement. The *in silico* BCF and BAF values are also plotted for comparisons to the reliable measured data. Collectively the data show consensus in the BCFs and BAFs for fish, recognizing that natural variability is expected, and all sources of uncertainty cannot be eliminated. The figure provides a nice method to arrange the data in a meaningful way for WoE evaluations. The BCF and BAF data for invertebrates is more variable, perhaps reflecting an absence of standardized testing methods for invertebrates and a more diverse range of biology.

Figure 11. Bioaccumulation data for fish and invertebrates used in problem statement 1 in comparison to "B" threshold criteria and associated reliability scores from 0 (Not Reliable/Critical Fail) to 5 (Most Reliable). BCF and BAF data on a wet weight basis. In silico data are plotted in the gray section for comparative purposes and are not assigned an RS in this figure. B=bioaccumulative; BAF=bioaccumulation factor; BCF=bioconcentration factor; nB=nonbioaccumulative; RS=reliability score; ww=wet weight. [Acknowledgement: Figure prepared by Liisa Toose].



A summary of the reliability scores for the BMF and TMF values for aquatic organisms in comparison to the threshold for determining bioaccumulation is shown in **Figure 12.** The reliability scores reflect the relative quality of the various LoE based on data evaluation methods derived from OECD Testing Guidance and expert judgement. The *in silico* BMF values are also plotted for comparison against the reliable measured data. Collectively, the data show consensus in the BMFs and TMFs, recognizing that natural variability is expected and all sources of uncertainty cannot be eliminated. The figure provides a nice method to arrange the data in a meaningful way for WoE evaluations. As noted above, the lab BMFs are generally lower than the field BMFs because they do not include co-exposure to the chemical in the water as occurs in the environment.

Figure 12. Biomagnification data for fish and invertebrates used in problem statement 2 in comparison to "B" threshold criteria and associated reliability scores from 0 (Not Reliable/Critical Fail) to 5 (Most Reliable). These are lipid-normalized BMFs and TMF data (C). In silico data are plotted in the gray section for comparative purposes and are not assigned an RS in this figure. B=bioaccumulative; BMF=biomagnification factor; nB=nonbioaccumulative; RS=reliability score; TMF=trophic magnification factor. [Acknowledgement: Figure prepared by Liisa Toose].



7.3.4 Summary -- PHE

PHE is a data rich chemical and evidence was available from *in silico, in vivo* and *in situ* sources. Nonetheless, both measured and modelled data have uncertainty associated with them. All LoE summarized in this case study have been subject to extensive data reliability (quality) evaluation using current methods. While only data considered of reliable quality were used in this case study, it must be recognized that uncertainty in the data still exists. Natural variability must also be recognized when looking at diverse data sets across various species.

The goal of this IATA is to provide a transparent and repeatable process for understanding and communicating the approach and methods that underpin bioaccumulation assessment. In keeping with this goal, the reliability scores for BCF, BAF, BMF, and TMF were determined using a transparent list of criteria largely developed from OECD TG for each data type. However, this process sometimes still requires informed and documented expert judgement. Furthermore, data integration and weighting of evidence followed approaches described herein and did not necessarily follow regulatory guidance for assessing B that may exist. Particularly for data rich chemicals, the steps of selection, critical evaluation, and weighting of selected data could lead to different conclusions regarding bioaccumulation assessment outcomes.

From **Tables 41 – 42** it is apparent that sufficient and consistent high-quality evidence is generally available for both problem statements. However, there is considerably more data and data types for PHE, compared

with the data poor examples. The available data show good coherence from *in silico* to *in situ* levels supporting a relatively overall strong evidence set for decision-making. Depending on the specific decision context and the required level of confidence, as an example of a very data rich chemical, the evidence as presented in this case study is likely sufficient to arrive at a regulatory conclusion for bioaccumulation and biomagnification (if applicable). For example, the strength of the evidence presented in this case study would, with this specific IATA data selection, suggest that PHE is not vB or B according to the criteria in Table 2, BCF in fish <5000, based on the criteria specified for problem statement 1 and nB according to the criteria in Table 2, and BMF or TMF <1, based on the criteria specified for problem statement 2. Other decisions could involve conducting full scale risk assessments and informing monitoring programs to ensure current trends remain in the environment and tissue residue levels do not approach levels of internal toxicity.

This case study should not be interpreted as an official regulatory decision made by the authoring member countries, because any jurisdiction may:

1. Select additional data that are not included in the case study and/or
2. Reach a different conclusion about the reliability or weighing of one or more individual studies, based on expert judgment and regulatory context.

All choices presented in the case study have not individually been agreed upon by all representatives of the OECD Working Party on Hazard Assessment. Different choices may lead to different scientific conclusions while applying the same approach.

Annex A. Abbreviations / Acronyms / Definitions

1.1 General Terms

ADME	Absorption Distribution Metabolism Excretion
B	Bioaccumulation
BAT	Bioaccumulation Assessment Tool
BET	Bioaccumulation Estimation Tool
DET	Data Evaluation Template
DHCP	Dicyclohexyl phthalate
EAS-E Suite	Exposure and Safety Estimation Suite
EC	European Commission
ECHA	European Chemicals Agency
GHS	Globally Harmonized System
IATA	Integrated Approach to Testing and Assessment
IOC	Ionizable Organic Chemical
LoE	Line(s) of Evidence
OECD	Organisation for Economic Cooperation and Development
OPERA	Open (Quantitative) Structure-activity/property Relationship App
PBT	Persistent, Bioaccumulative, Toxic
PHE	Phenanthrene
POP	Persistent Organic Pollutant
PPLFER	Poly-Parameter linear free energy relationship
QSAR	Quantitative Structure-Activity Relationship
REACH	Registration, Evaluation, Authorisation and Restriction of Chemicals
SVHC	Substances of Very High Concern
TBECH	1,2-Dibromo-4-(1,2-dibromoethyl)cyclohexane
TK	Toxicokinetics
TSCA	Toxic Substances Control Act
UK	United Kingdom
UNEP	United Nations Environment Programme
USEPA	United States Environmental Protection Agency
UVCB	Unknown or Variable Composition, Complex Reaction Products and Biological Materials
WoE	Weight of Evidence

1.2 Partition Coefficients / Distribution Ratios

Partition coefficient (or ratio)	The equilibrium distribution of a neutral organic chemical (or the neutral form of an ionizable organic chemical, IOC) between two defined phases (e.g., octanol and water)
Distribution ratio	The weighted average of the partition coefficient of the neutral form of an IOC (e.g., $K_{OW,N}$) and the apparent partition coefficient of the charged form(s) (e.g., $K_{OW,I}$) based on the dissociation constant(s) of the chemical and the pH of the aqueous phase
K_{OW}	Octanol-water partition coefficient (or ratio)
K_{AW}	Air-water partition coefficient (or ratio)
K_{OA}	Octanol-air partition coefficient (or ratio)
K_{SW}	Storage lipid-water partition coefficient (or ratio)
K_{MW}	Membrane-water partition coefficient (or ratio)
K_{PW}	Protein (structural)-water partition coefficient (or ratio)
K_{BSA}	Bovine serum albumin-water partition coefficient (or ratio)

1.3 *In Vitro* Biotransformation Assays and *In Vitro-In Vivo* Extrapolation (IVIVE)

k_e	First-order rate constant for in vitro biotransformation, derived from the slope of the depletion curve from the test (or inferred from confirmed product formation)
$t_{1/2}$	First-order half-life for in vitro biotransformation, derived from the slope of the depletion curve from the test ($t_{1/2} = \ln(2)/k_e$)
$CL_{IN\ VITRO,INT}$	Intrinsic clearance in the in vitro test system, normalized to the type of biological material present (e.g., per mg S9 or microsomal protein, per 10^6 liver cells)
$CL_{IN\ VIVO,INT}$	In vivo intrinsic clearance extrapolated from in vitro test data ($CL_{IN\ VITRO,INT}$) and normalized to body weight
CL_H	Hepatic clearance (accounting for possible blood flow limitation)
$f_{U,i}$	Unbound fraction in medium *i*, where *i* can be blood (Bl) or plasma (P) or the aqueous phase of an in vitro S9 (S9), hepatocyte (HEP) or microsomal system (MIC)
f_U	Ratio of unbound (freely-dissolved) fraction of the chemical in blood or plasma and an in vitro test system (e.g., $f_{U,Bl} / f_{U,S9}$)

1.4 *In Vivo* Bioaccumulation Parameters

k_1	Respiratory uptake rate constant (i.e., from water for aquatic organisms and for air for mammals)
k_D	Dietary uptake rate constant
k_2	Respiratory elimination rate constant
k_E	Fecal egestion rate constant
k_B (aka k_M or k_{MET})	Whole-body biotransformation rate constant
k_G	Growth dilution rate constant
k_T	Total (terminal) elimination rate constant
$k_{B,N}$	Whole-body biotransformation rate constant, normalized to a standard mass
HL_B	Whole-body biotransformation half-life

$HL_{B,N}$	Whole-body biotransformation half-life, normalized to a standard mass
HL_T	Total (terminal) elimination half-life
$HL_{T,N}$	Total (terminal) elimination half-life, normalized to a standard mass and temperature

1.5 Bioaccumulation Metrics (General)

B	Bioaccumulative
nB	Not Bioaccumulative
vB	Very Bioaccumulative
BCF	Bioconcentration factor (L/kg) = C_{Biota} / C_{Water}
BAF	Bioaccumulation factor (L/kg) = C_{Biota} / C_{Water}
BMF	Biomagnification factor (kg/kg) = C_{Biota} / C_{Diet}
TMF	Trophic magnification factor

1.6 Laboratory BCF

Lipid-standardized (BCF)	Lipid-standardization = conversion of a wet-weight BCF for a given lipid content (e.g., 3.5%) to the wet-weight BCF expected for a 5% lipid content fish. In this example, the BCF of the 3.5% lipid content fish would be multiplied by a factor of ~ 1.4 (0.05/0.035)
k_1	Gill uptake rate constant
k_T	Total depuration (elimination) rate constant
k_G	Growth dilution rate constant
BCF_{SS}	Steady-state bioconcentration factor
$BCF_{SS,L}$	Steady-state bioconcentration factor, wet-weight, standardized to a 5% lipid content fish
BCF_K	Bioconcentration factor, derived using kinetic data (k_1/k_T)
$BCF_{K,G}$	Growth-corrected kinetic BCF
$BCF_{K,L}$	Kinetic BCF, wet-weight, standardized to a 5% lipid content fish
$BCF_{K,G,L}$	Growth-corrected kinetic BCF, wet-weight, standardized to a 5% lipid content fish

1.7 Laboratory BMF

Lipid-normalized (BMF)	Lipid-normalization = conversion of a BMF based on wet-weight concentrations in the predator and its diet to a BMF based on the assumed concentrations of the chemical in the lipids of the predator and its diet (e.g., $C_{Pred,L} = C_{Pred} / L_{Pred}$, where C_{Pred} is the wet-weight concentration and L_{Pred} is the total lipid content of the predator). The BMF can also be expressed as chemical fugacity or activity ratios, e.g., (Arnot 2022).
I	Food ingestion rate (normalized to mass of organism)
BMF_G	Growth-corrected BMF
BMF_L	Lipid-normalized BMF
$BMF_{L,G}$	Lipid-normalized, growth-corrected BMF

Annex B. Summary of Regulatory Criteria Related to Bioaccumulation Assessments

Table A B.1. Summary of Regulatory Criteria related to Bioaccumulation Assessments.

Jurisdiction	Legal Instrument	References
European Union	Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH)	(European Commission 2006; ECHA 2017a)
United Kingdom	UK Registration, Evaluation, Authorisation and Restriction of Chemicals (UK REACH)	(ECHA 2017a; UK Government 2021)
United States	Toxic Substances Control Act	(US EPA 1976; 2016)
Canada	Persistence and Bioaccumulation Regulations	(Government of Canada 2000)
Japan	Chemical Substance Control Law	(METI 1973; 2008)
Australia	Industrial Chemicals Act	(Australian Government 2019, 2020)
United Nations	UN Stockholm Convention	(UNEP 2001; 2019)

Annex C. Data Evaluation Template

Table A C.1. DET for Gemmill et al (2011) TBCEH BMF study. Reliability score = 4.53 / 5.

Quality Criteria for Data Reliability of a fish BMF Study

1. Were the BMF units clearly reported (e.g., kg/kg wet weight)?	(•) Yes	( ) No		
2. BMF for parent chemical reported	(•) Yes	( ) No		
3. If BMF was calculated as Cfish/Cdiet, was the steady state assumption (+/- 20%) confirmed?	(•) Yes	( ) No	( ) N/A	
4. If BMF was calculated as (I·ED)/kT, were the rate constants with units clearly reported?	(•) Yes	( ) No	( ) N/A	
5. Organism concentration measured directly for chemical of interest	(•) Yes	( ) No		
6. Dietary uptake efficiency (ED or alpha) <= 100%?	(•) Yes	( ) No		
7. For ionising chemicals: was pH reported and within 0.5 log units of average?	pH 6 - 8.5 ☐	<> 0.5 log mean ☐		
8. Diet concentration measured directly for chemical of interest?	☑			
9. Diet lipid content reported?	☑			
10. Was growth rate reported?	☑			
11. Mortality/adverse effects in test/control group < 5%	(•) Yes, both	( ) No, ctrl	( ) No, test	
12. Whole body fish lipid content reported	(•) Yes	( ) Partial	( ) No	
13.Test species reported? [Check OECD species]	( ) Yes, OECD	(•) Yes, not OECD	( ) No	
14. Fish mass reported? Yes, Partial (start or end) or No	(•) Yes	( ) Partial	( ) No	
15. Whole body of fish analyzed? Tissue: [Whole Body] Lipid %: [* / +]	(•) Yes	( ) Partial	( ) No	
16. Feeding rate reported in the range of 1-3% body weight per day?	☑			
17. Was there a control group?	☑			
18. What was the chemical purity?	>=98% (•)	>=95% ( )	<95% ( )	NR ( )
19. LOQ reported?	☑			
20. Conducted according to recognized international standard, e.g. OECD?	( ) Yes	(•) with some mods.	( ) No	
21. Study consistent with GLP or guiding principles?	( ) Yes	( ) with some mods.	(•) No	
22. Test design	flow through (•)	semi-static ( )	static ( )	NR ( )
23. Water temperature reported AND appropriate for species AND constant (+/- 2C)	☑			
24. Diet concentration < 1% reported acute toxicity?	☑			
25. For neutrals: was pH reported and within 0.5 log units of average?	pH 6 - 8.5 ☑	<> 0.5 log mean ☐		

26. Was dissolved oxygen reported and > 60%?	☑	29. Minimum of 4 fish per sampling event?	☑
27. All organisms in study of similar size(+/- ~30% wt. or vol.)?	☐	30. Water hardness is reported AND 10-250 mg/L?	☑
28. Acclimatization for at least 14 days under test conditions?	☑	31. Light-dark cycle reported AND 12-16 h illumination (or otherwise appropriate?)	☑

Annex D. Brief summary of studies not considered in Section 7.3 (Phenanthrene case study) due to study reliability issues (reliability score of 0)

The critical review by Armitage et al., (2021) included the compilation and critical evaluation of 74 measured *in vivo* LoE for fish and invertebrate species from laboratory and field studies. All laboratory BCF and BMF data for aquatic species used in this example were critically evaluated and provided in the Supplemental Data sections of Armitage et al., (Armitage 2021). Studies with reliability scores of 0 (critical fail) from Armitage et al., (Armitage 2021) were not included in the WoE previously or for this case study. For additional detailed information on the data quality evaluation (including identified study limitations), and calculation of reliability scores, please see (Armitage 2021).

Reference	B-metric	Organism	SS/K	Value(s)	Identified Study Limitations
(Carlson 1979)	Lab Fish BCF	Fathead minnow	SS	6024	Steady-state not achieved; water concentration not stable throughout the experiment; reported water concentrations = 1.1 – 3.3 µg/L; mixture of PHE various other PAHs; evidence of induction; water and fish concentrations varied substantially
				3222	
				2388	
				2206	
				1965	
(Cheikyula 2008)	Lab Fish BCF	Red sea bream	SS	141	Mixture (4 PAHs) in seawater with 2 compounds ≥ freshwater solubility
(Burkhard 2012)	Fish Field BAF	Various	SS	15	5-year gap between collection of fish tissue and water samples; ambiguous reporting of lipid content of tissue; fish concentrations close to analytical LOD
(Khairy 2014)	Fish Field BAF	*Lepomis gibbosus*	SS	4660	Very high BAF values reported are inconsistent with the lack of biomagnification by the TMF of the same study
		Fundulus diaphanus		1069	
		Leopmis macrochirus		3228	
		Hybognathus regius		4234	
		Esox americanus		1815	
		Fundulus diaphanus		5755	
		Morone americana		4467	
		Anguilla rostrata (11-12 cm)		5933	
		Anguilla rostrata (28-110 cm)		4337	
		Hybognathus regius		19198	

Reference	B-metric	Organism	SS/K	Value(s)	Identified Study Limitations
		Morone saxatilis (18-20 cm)		4723	
		Morone americana		9926	
		Morone saxatilis (9.6-10.4 cm)		3490	
		Morone saxatilis (20-33 cm)		1781	
		Hybognathus regius		16020	
		Dorosoma cepedianum		17226	
		Fundulus heteroclitus		19020	
		Menidia menidia (2.2-3.6 cm)		11260	
		Menidia menidia (7.8-9.4 cm)		18181	
(Landrum 1988a)	Lab Invert BCF	*Pontoporeia hoyi*	K	5600	Kinetic BCF; k_1 and k_T were not reported clearly (units unclear)
(Landrum 2003)	Lab Invert BCF	*Diporeia spp*	K	6996	Could not determine whether organism concentration not measured directly. Water concentration was not measured directly. Very little study information.
(Cailleaud 2009)	Lab Invert BCF	*Eurytemora affinis*	SS	124	Mixture - although all components appear to be < individual water solubilities, enzyme induction due to exposure scenario is a possibility. Dry wt concentrations reported; Assumed dry/wet = 0.3 and lipid/dry =0.2.
(Landrum 1988b)	Lab Invert BCF	*Hexagenia limbata*	K	411	Kinetic BCF; k_1 and k_T were not reported clearly. Organism concentration not measured directly.
				2005	
(Khairy 2014)	Field Invert BAF	*Callinectes sapidus*	SS	5.9e4	Very high BAF values reported here are inconsistent with the lack of biomagnification by the TMF of the same study; Given partitioning properties of PHE, body of evidence suggests that dietary uptake is unlikely to be significant.
(Moermond 2007)	Field Invert BMF	*"Zooplankton"*	SS	1.64	Standard deviation of the zooplankton BMF = ±1.4

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Case study on the Use of Integrated Approaches for Testing and Assessment (IATA) for Bioaccumulation - Ninth Review Cycle (2023)

Series on Testing and Assessment No. 404

The objective of the Integrated Approaches for Testing and Assessment (IATA) Case Studies Project is to increase experience with the use of IATA by developing case studies which constitute examples of predictions that are fit for regulatory use. The aim of this project is to create common understanding of using novel methodologies and the generation of considerations/guidance stemming from these case studies. This case study was developed by the Health and Environmental Sciences Institute (HESI), Canada, United Kingdom, United States, Business at OECD (BIAC) for illustrating practical use of IATA and submitted to the 2023 review cycle of the IATA Case Studies Project. This IATA provides examples to aid evaluators in the collection, generation, evaluation, and integration of multiple lines of evidence (LoE) for clear and transparent decision-making within defined problem contexts. This IATA includes guidance for data collection and generation from publicly available databases and models that can be readily used for data poor and relatively data rich chemicals. Three illustrative case studies representing both data poor and data rich chemicals are presented to illustrate the applicability of the IATA for Bioaccumulation assessment.