The Bioaccumulation Assessment Tool (BAT)

Version 2.02

USER MANUAL

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Arnot Research & Consulting

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Key Terminology/Acronyms/Definitions

BAT Terminology

BAT	Bioaccumulation Assessment Tool
DET	Data Evaluation Template
LoE	Line(s) of Evidence
WoE	Weight of Evidence

Partition Coefficients/Distribution Ratios

Partition coefficient	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
K_{AW}	Air-water partition coefficient
K_{OA}	Octanol-air partition coefficient
K_{SW}	Storage lipid-water partition coefficient
K_{MW}	Membrane-water partition coefficient
K_{PW}	Protein (structural)-water partition coefficient
K_{BSA}	Bovine serum albumin-water partition coefficient
K_{BW}	Biota-water partition coefficient
K_{BA}	Biota-air partition coefficient
K_{POC}	Particulate organic carbon-water partition coefficient
K_{DOC}	Dissolved organic carbon-water partition coefficient
D_{OW}	Octanol-water distribution ratio
D_{AW}	Air-water distribution ratio
D_{OA}	Octanol-air distribution ratio
D_{SW}	Storage lipid-water distribution ratio
D_{MW}	Membrane-water distribution ratio
D_{PW}	Protein (structural)-water distribution ratio
D_{BSA}	Bovine serum albumin-water distribution ratio
D_{BW}	Biota-water distribution ratio
D_{BA}	Biota-air distribution ratio
D_{POC}	Particulate organic carbon-water distribution ratio
D_{DOC}	Dissolved organic carbon-water distribution ratio
spLFER	Single parameter linear free energy relationship
ppLFER	Polyparameter linear free energy relationship
QSAR	Quantitative Structure-Activity Relationship

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., $f_{U,Bl} / f_{U,S9}$)
P_{BlW}	Blood-water partition coefficient (i.e., sorption capacity of whole blood relative to water)
BCF_P	Partitioning-based bioconcentration factor (i.e., sorption capacity of whole-body relative to water, also denoted K_{BW})
Q_C	Total cardiac output
Q_H	Blood flow to liver
LW	Liver weight as fraction of total body weight (i.e., kg liver / kg body weight)
LF	Fraction of total cardiac output flowing to liver
$V_{D,Bl}$	Volume of distribution of the chemical, referenced to blood

In Vivo Bioaccumulation Parameters

k_1	Gill uptake rate constant
k_D	Dietary uptake rate constant
k_2	Gill elimination rate constant
k_E	Fecal egestion rate constant
k_B (aka k_M or k_{MET})	Biotransformation rate constant
k_G	Growth dilution rate constant
k_T	Total depuration (elimination) rate constant
$k_{B,N}$	Biotransformation rate constant, normalized to a standard mass and temperature
HL_B	Biotransformation half-life
$HL_{B,N}$	Biotransformation half-life, normalized to a standard mass and temperature
HL_T	Total elimination half-life
$HL_{T,N}$	Total elimination half-life, normalized to a standard mass and temperature

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

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

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). BAT considers all biological components (lipids, proteins) when presenting "lipid-normalized" values.
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
E_D (α)	Gut uptake efficiency (chemical)
K_{BG}	Body-gut partition coefficient
K_{DG}	Diet-gut partition coefficient
BMF_{MAX}	Theoretical maximum BMF (excluding biotransformation)

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Preface

The BAT Ver.2.02 will only function properly on computers using a Windows operating system. The computer must use the period (.) as the decimal separator rather than the comma (,) to ensure accurate results. Reconfiguration guidance is provided in Appendix A1 (Windows Settings to Ensure Proper Functioning of the BAT). The Quick Start Guide that accompanies this User Manual provides explicit step-by-step instructions for operating the BAT. The Quick Start Guide can be considered to expedite the use of the BAT; however, users are encouraged to read the full User Manual before using the BAT. Please check for the latest version at www.arnotresearch.com. If you "crash the BAT", please close the file (the results may not be reliable) and report the issue using the BAT response form at www.arnotresearch.com.

Updates in BAT version 1.01

Updates to BAT output:

☑ Checks to see if study reliability would like to be checked again on all study entry sheets
☑ Field TMF studies can be critically failed now
☑ Control length of report references page
☑ Benchmarking plots only show studies with reliability AND relevance > 0
☑ Results and Report summary sheets: Lab BMF non-growth corrected value output correctly
☑ Chemical Summary-IOC summary of HLAW_T(medium) now correctly output

Updates to BAT calculations:

☑ Biotransformation weighted average calculation starts on correct line
☑ Zfish for ionics calculation - adjustment for pH

Updates in BAT version 2.0

Since the initial release of BAT Ver.1.0 (October 2018), many updates have been undertaken. These changes can be summarized in seven general areas: (i) workflow, interface and increased capacity for pre-existing lines of evidence, (ii) new organisms for additional lines of evidence (i.e., invertebrates and laboratory/aquatic mammals), (iii) improved treatment of bioenergetics in the built-in BAT food web bioaccumulation models, (iv) consideration for chemical degradation in the gastro-intestinal tract (GIT) for modelled vertebrate species, (v) new summaries for reported biotransformation and dietary absorption efficiency (E_D) data using weighted means, reliability scores and confidence factors to characterize these processes in the food web models, (vi) updated and improved consistency of the *in vitro* Data Evaluation Templates (DETs), and (vii) other corrections/updates.

(i) Workflow, Interface, and Increased Capacity

- New user option to enter chemical-specific enthalpies of phase change to improve temperature correction of chemical partitioning.
- Increased capacity to allow users to include more LoE for each biotransformation study type and B-metric. A limit of five per study type was imposed in BAT Ver.1.0; this has been increased to twenty-five per study type in BAT Ver.2.0.
- Addition of a worksheet "Work Area" for copying and pasting sources, calculating required units or for any other useful information to the assessment.
- Addition of a "Work Area" on the various study input sheets.
- To avoid the possibility of pseudo-replication influencing the WoE, there is now a "pop-up" to guide the selection of a single B-metric from the available predicted or entered values for each study.

- An improved workflow with greater flexibility to change or add physical-chemical properties or biotransformation rate information after BAT has summarized and integrated the various LoE into a WoE. On making these changes, however, the "*Add to BAT*" button must be "clicked" on each previously entered study to update the results and the "*Next*" button on the **BAT Main** sheet must be "clicked" to generate updated results.
- Improved user flexibility in sorting the data, reassigning relevance and/or thresholds to B-metric types on the **Summary Results** sheet.

(ii) New Organisms and Revised Food Web Bioaccumulation Models

Aquatic Invertebrates

- New input sheets for select aquatic invertebrates for laboratory tests.
- New Data Evaluation Templates (DETs) for select aquatic invertebrates for laboratory tests.
- Revised existing input sheets for "B" field data derived from aquatic invertebrates.
- Revised existing DETs to assess reliability of "B" field data derived for select aquatic invertebrates.
- New "B" models for representative aquatic invertebrate taxa to simulate laboratory experiments and field bioaccumulation.
- New "B" data for aquatic invertebrates into fugacity ratio calculations and summary output.
- New "B" data for aquatic invertebrates into the BAT WoE calculations and summary output.

Mammals

- New input sheets in the BAT for laboratory toxicokinetics (TK) tests using rodents.
- New DETs for laboratory TK tests using rodents.
- Revised existing input sheets for "B" field data derived for terrestrial and aquatic mammals.
- New "B" models for rat and mouse to simulate laboratory experiments.
- Added a "B" model for a seal as a representative upper-trophic level aquatic mammal in the field environment.
- Included new "B" data for terrestrial and aquatic mammals in fugacity ratio calculations and summary output.
- Included new "B" data for terrestrial and aquatic mammals into the BAT WoE calculations and summary output.

(iii) Bioenergetics

- Improved energy and water balance for fish and mammals in the built-in BAT food web models.

(iv) Chemical Degradation in the GIT

- Incorporated the mechanistic chemical dietary uptake efficiency (E_D) model for vertebrates (fish and mammals) described in Arnot and Mackay 2018 [1], whereas the E_D model for invertebrates is based on the model described in Arnot and Gobas 2004 [2].
- Added a new user option to enter empirical E_D values to replace the default model E_D calculations for vertebrate species. This includes a new E_D input sheet to record study information and an associated DET by which the reliability of this information is assessed. If multiple empirical E_D values are entered for each organism type (e.g., "fish" and "herbivore" and "omni-/carnivores"), the weighted geometric mean is calculated and used preferentially over the default E_D model calculations used in the built-in BAT model B metric calculations. The weighting is based on study-specific reliability scores. The mean value is used in the calculation of the uptake of chemical

through the GIT and a confidence factor (CF) characterizing the uncertainty in this parameter is used to propagate this uncertainty in the built-in BAT model calculation output.

(v) Summarizing Biotransformation Rates using Reliability Scores

- If multiple biotransformation rate constant (or half-life) values for each organism type (e.g., "fish" and "mammal") are available, a weighted geometric mean of the biotransformation half-life (HL_B) is calculated and used in the built-in BAT model B metric calculations for each organism type. The weighting is based on both study type (in vitro, in vivo, in silico) and study-specific reliability scores. Uncertainty in HL_B is reflected in a confidence factor (CF) and this uncertainty is propagated into the built-in BAT model calculations providing lower and upper range estimates.

(vi) In Vitro Biotransformation, IVIVE, and Data Evaluation Template Updates

- Added a new user option to select a "Composition-based" approach to estimate fractions unbound in *in vitro* biotransformation assays ($f_{U,assay}$), whereby $f_{U,assay}$ is calculated as a function of assay composition (storage lipid, membrane lipid, protein and water content) and partitioning data.
- In vitro data reliability forms and scoring have been updated.

(vii) Other Updates and Corrections

- Correction: Zdiet for mammals consuming fish now considers both the neutral form and charged form of IOCs.
- Fugacity ratio calculations updated and determined separately for steady-state or kinetic LoE.
- Temperature corrections to environmental and biological partition coefficients for neutral and ionic chemicals occur throughout the BAT based on the built-in BAT model calculations ("BAT-Estimated") and reported (study) organism and environmental temperatures.
- All mammals and birds are deemed "homeotherms-herbivore" or "homeotherms-omni/carnivore" based on their diet.
- Updated graphical output to accommodate the new classes of organisms (as above).
- Output of total half-life of each output B-metric/organism on a new output sheet "HLT".
- Refinement to input sheet indications for "required for calculation" inputs on all forms.

Updates in BAT version 2.02

The built-in toxicokinetic, bioaccumulation and food web bioaccumulation models in BAT Ver.2.02 have been updated to better reflect the bioenergetics and system conditions (i.e., temperatures) in aquatic ecosystems and laboratory environments. To address current data gaps, a significant update in Ver.2.02 includes the option for users to scale biotransformation rate constants from fish to aquatic invertebrates with user-entered scaling factors, otherwise default scaling factors are initially assumed. The user can now also choose the relative "importance" (weighting coefficients) of in vivo, in vitro and in silico biotransformation rate constant (or half-life) data used to calculate the geometric mean and the associated confidence factors (CF), otherwise default weighting coefficients are initially assumed. The CF also considers reliability scores for each LoE (as previously implemented in Ver.2.0). The geometric mean value for the biotransformation rate constant is then used to parameterize the built-in BAT bioaccumulation models. The CF values are used to propagate uncertainty in biotransformation rate estimates as "upper" and "lower" BAT model calculated B-metrics. The user may now choose whether to summarize Weight of Evidence results in terms of either growth-corrected or non-growth corrected lipid-normalized (or standardized) B-metrics for laboratory fish and rats. Finally, there are a few important "bug fixes" from Ver.2.0 that are listed in the Quick Start document, including a correction of units from hours to days on the total half-life (HLT) output sheet.

Introduction

Bioaccumulation is the net result of competing rates of chemical uptake into, and elimination from, an organism [3]. Bioaccumulation is often quantified using bioconcentration factors (BCFs) measured with fish in laboratory settings [4]. The BCF represents uptake via respiratory and dermal exchange only [3]. In the environment organisms are also exposed to chemicals in their diet. For aquatic organisms, the bioaccumulation factor (BAF) represents exposure and uptake from all possible routes, i.e., the surrounding environment and the diet. Dietary uptake is an important exposure route for hydrophobic compounds [5-7]. The relationship between dietary exposures and bioaccumulation can be evaluated using laboratory biomagnification factors (BMFs) [4] and field BMFs [8] and food web trophic magnification factors (TMFs) [9]. In the absence relevant bioaccumulation data, the octanol-water partition coefficient (K_{OW}) is often considered for screening assessments as a surrogate for organism-water partitioning; however, K_{OW} neglects key biological processes, most notably biotransformation.

Traditionally, bioaccumulation assessment has focused on aquatic organisms and ecosystems only; however, research has shown the fundamental differences in bioaccumulation potential between water-ventilating and air-breathing species [10-13]. In recent years additional lines of evidence and criteria for air-breathing organisms are being considered, e.g., the octanol-air partition coefficient (K_{OA}). The concept of applying fugacity ratios in a weight of evidence approach for assessing bioaccumulation, more specifically biomagnification, has been proposed [8]. The total chemical elimination half-life from an organism (HL_T) has also been proposed to assess bioaccumulation [14, 15]. The biotransformation rate constant (k_B) is a key determinant of net bioaccumulation, particularly for chemicals with higher inherent bioaccumulation potential (i.e., hydrophobic chemicals) because of elevated partition coefficients [3, 16, 17]. The biotransformation rate is a well-recognized process mitigating bioaccumulation of the parent compound in experimental laboratory test data, e.g., [17, 18] and field exposures, e.g., [19, 20].

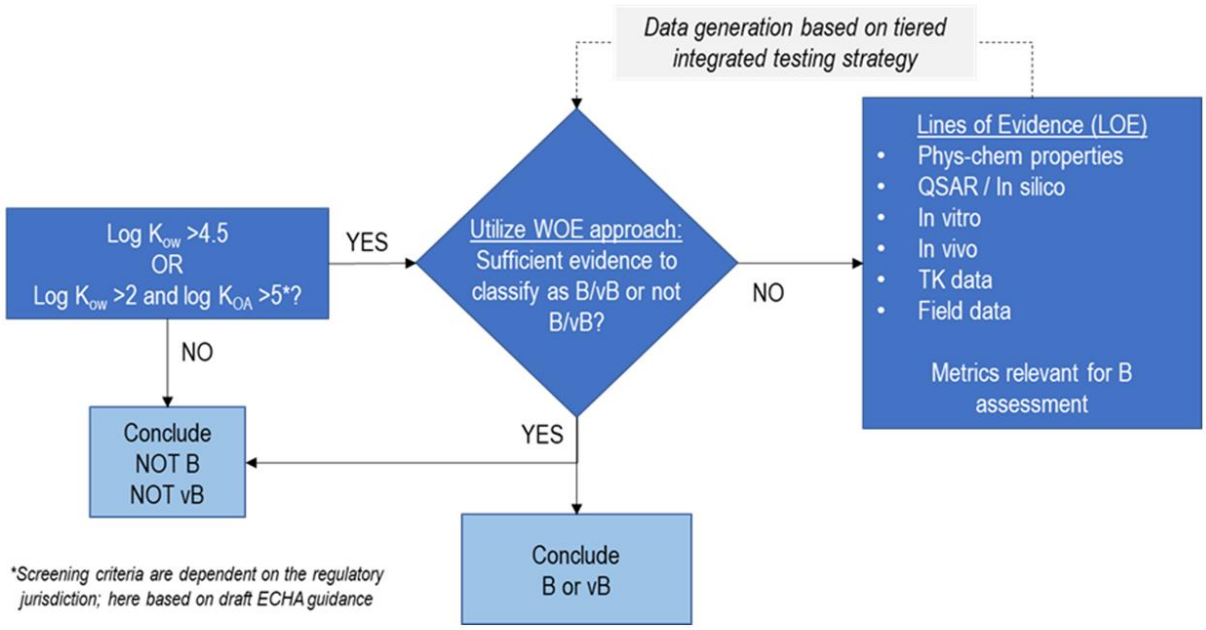
Chemicals are undergoing Bioaccumulation ("B") hazard assessment as part of national and international regulatory programs and treaties [21-25]. Methods and criteria for "B" assessments often vary between jurisdictions. Bioaccumulation assessment can be a scientific and regulatory challenge in some cases because there are various metrics for assessing bioaccumulation in aquatic and terrestrial organisms and food webs (e.g., BCF, BMF, BAF, TMF), various "B" criteria (threshold values for "B" classification), variability and uncertainty in bioaccumulation data, and sometimes conflicting "B" classification results. A weight of evidence (WoE) approach is commonly recommended in most regulatory programs (e.g., REACH Annex XIII). 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 Lines of Evidence (LoE) for 'B' assessment decision-making.

Figure 1 illustrates the general flow of information and the decision-making process for B assessment in the context of REACH regulations (Annex XIII). At the core of this process is the "decision-making diamond" in the middle that seeks to integrate various LoE in a WoE approach, the crux of Annex XIII. Current assessments can be subjective, inconsistent, and unclear because of the vague language in Annex XIII and the necessarily subjective nature of professional judgement. An organizational framework is required to integrate various LoE (bioaccumulation data) and the best available science to guide decision-making.

For "B" hazard assessment, LoE can include various bioaccumulation metrics that are currently used in assessment programs (i.e., BCF, BAF, BMF, TMF). The LoE can be obtained from laboratory or field measurements or from in silico (model) calculations, or from a combination of in vitro measurements and in silico (model) calculations. Standardized test guidelines have been developed for measuring laboratory BCFs and BMFs in fish [4] and for measuring in vitro biotransformation rates [26-28]. Mechanistic

bioaccumulation, i.e., toxicokinetic (TK), models have successfully been developed to quantify the bioaccumulation of organic chemicals in aquatic and terrestrial wildlife and humans [2, 10, 12, 13, 15, 29-31]. These models simulate chemical uptake and elimination within an organism, or when combined, for various organisms in a food web. Quantitative Structure-Activity Relationships (QSARs) are also commonly employed for predicting bioaccumulation endpoints, e.g., for predicting fish BCFs [32-37] and BAFs [7, 38] and for biotransformation half-lives [39-43]. Many bioaccumulation QSARs have been developed following OECD guidance [44, 45].

Figure 1. A general workflow for bioaccumulation assessment, demonstrating the intended purpose for the BAT

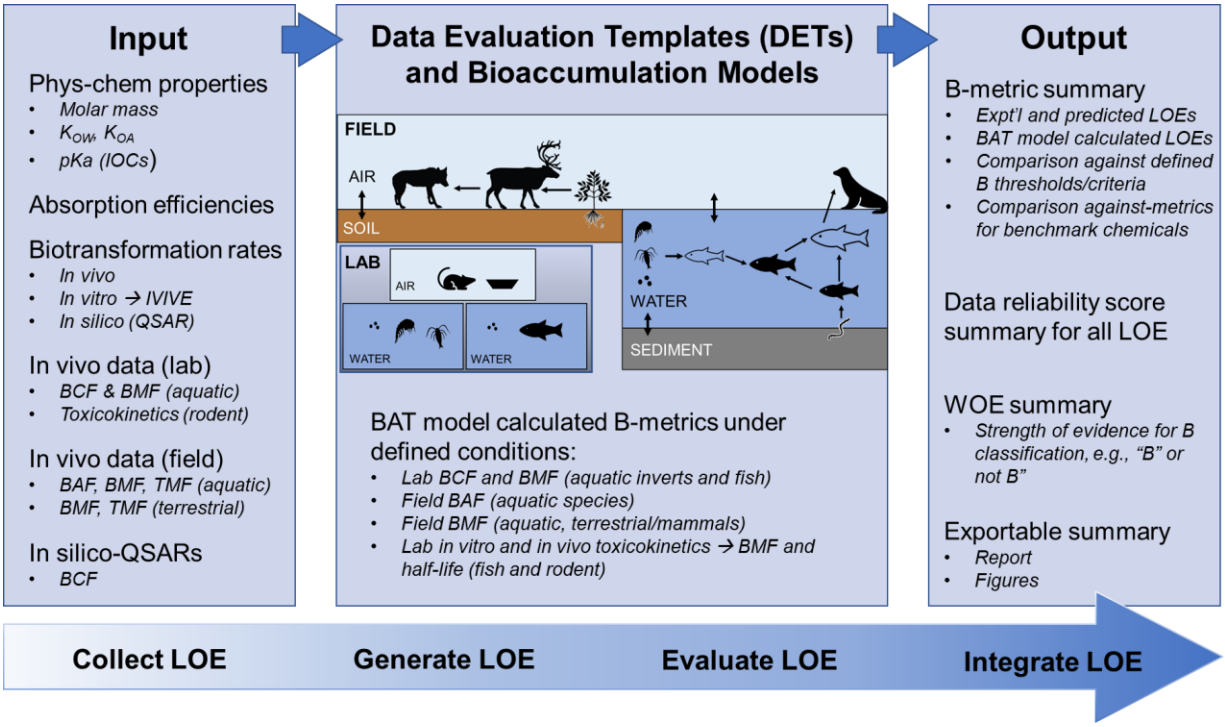


Main Objectives of the BAT Project

- Develop a user-friendly, spreadsheet-based tool to collect, generate, evaluate, and integrate various LoE (in silico, in vitro, in vivo) relevant for B assessment into a consistent and transparent Weight of Evidence (WoE) to aid decision-making. The user is responsible for documenting rationales for their selection of information. Figure 2 illustrates key conceptual elements of the BAT for evaluating chemicals in aquatic and terrestrial organisms and ecosystems. The current focus of the BAT is on relatively well-established model organism classes, i.e., fish and mammals, and to integrate relatively well-established or recently emerging data sources.
- Provide a framework to evaluate and potentially guide the development of information in a tiered, integrated manner:
  - Critically evaluate available data (measured and predicted) to inform B assessment.
  - Address uncertainty by following a tiered, integrated testing strategy.
- The BAT is developed primarily for collecting, evaluating, and incorporating various lines of evidence to aid B assessment decision-making following a WoE approach. However, it can also be

used in data poor situations to generate (predict) common lab and field B assessment metrics for representative aquatic and terrestrial species using chemical properties and estimates of biotransformation half-lives. It can also provide insights into key properties and processes relating to TK and bioaccumulation of organic chemicals for a range of species.

Figure 2. Conceptual diagram of the BAT (aquatic organisms and air-breathing organisms)



- The BAT **DOES NOT**:
  - Link directly to databases for operation; the user must gather information.
  - Conduct statistical analysis of the data (BCF, BMF, in vitro biotransformation rate, etc.); it is assumed that the user has a certain amount of information or has performed these analyses.
  - Supplant regulatory review of technical guidance.
  - Make decisions; the user must still make decisions based on the WoE provided by the BAT.
  - Consider possible metabolites; the BAT only performs parent chemical evaluations, or "one-chemical at a time"; metabolites could be considered with the BAT in a separate assessment.

The BAT Weight of Evidence Approach

Weight of Evidence (WoE) is a process of assembling, evaluating, weighing, and integrating evidence to come to a scientifically defensible conclusion and the WoE approach is used when scientific questions can only be answered by using multiple **Line(s) of Evidence** (LoE) [46]. The WoE approach provides a consistent framework for decision-making and needs to be transparent. *The WoE approach in BAT follows OECD "Guiding Principles and Key Elements for Establishing a Weight of Evidence for Chemical Assessment" [47].* The common elements for a QWOE approach include **LoE**, **Relevance Weighting**, **Reliability** and **Strength of Evidence** [46]. LoE in BAT include bioaccumulation metrics that are currently used in most

bioaccumulation assessment programs (i.e., BCF, BAF, BMF, TMF). LoE can be obtained from field data (e.g., BAF, BMF, or TMF) or laboratory data (e.g., BCF or BMF) or in silico (model) calculations (e.g., QSARs) or from a combination of in vitro measurements and in silico (TK model) calculations (e.g., in vitro biotransformation rates used to calculate BCFs). Each LoE is assigned a relevance weight (from 0 to 5) by the user *a priori*, though this can be updated later in the assessment, if necessary. The LoE are subject to data quality evaluations to determine reliability scores (from 0 to 5). The Strength of Evidence is determined by the frequency of "B" classifications based on all LoE. For example, if all LoE result in a "nB" classification the Strength of Evidence for the chemical being "nB" is 100% and the Strength of Evidence for the chemical being classified as "B" or "vB" is 0%.

Applicability Domain

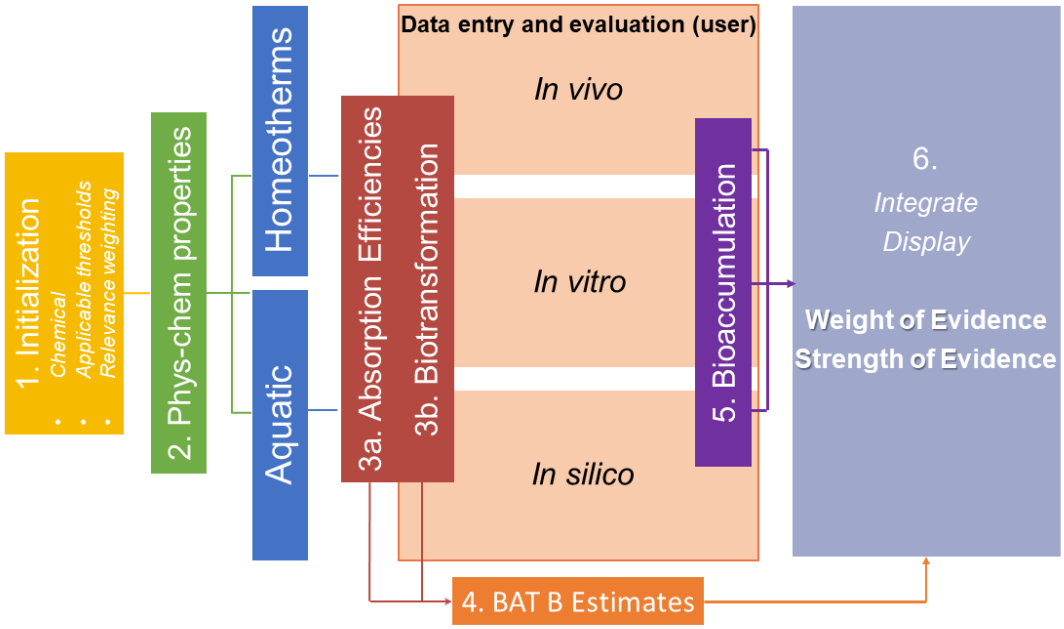
The BAT calculates bioaccumulation metrics such as lab BCFs, BMFs, as well as field BMFs and BAFs. Such metrics are labelled "in silico-based" in the BAT output summary. It is difficult to explicitly define the BAT-calculated applicability domain (AD). In general terms, the confidence in the BAT-calculated metrics reflects the general confidence and current state of knowledge for B assessment. The underlying theory, data and mechanistic knowledgebases are generally more developed for neutral organic chemicals than they are for ionizable organic chemicals (IOCs). For example, neutral organics with log K_{OW} from 1 to 8 have relatively more high-quality experimental data and technical understanding related to processes of bioaccumulation in fish than other organics such as appreciably ionized, or very hydrophobic (log K_{OW} > 8) organics and processes in other species, (e.g., autotrophs, invertebrates). Most bioaccumulation knowledge for mammals is available primarily from pharmaceutical sciences using rodent models and to some extent veterinary sciences. The built-in BAT model calculated metrics are often very sensitive to the physical-chemical partitioning (distribution) properties and the biotransformation rates (half-lives). For this reason, uncertainty in biotransformation rate estimates entered by the user are propagated into BAT-calculated B metrics. In other words, the more reliable the chemical information is, the more reliable the BAT-calculated metrics are expected to be, particularly for neutral organics with log K_{OW} from 1 to 8. The BAT-calculated metrics *only consider passive uptake and elimination processes*; active transport processes, such as chemical reabsorption in the kidney, are not included. This limitation is relevant when comparing BAT-calculated output against empirical bioaccumulation metrics for persistent chemicals subject to active TK processes, e.g., certain perfluorinated chemicals. The BAT models for in silico outputs are intended to evolve as the science evolves.

BAT is only applicable to discrete organic chemicals with biota-water (K_{BW} or D_{BW}) AND biota-air (K_{BA} or D_{BA}) partition coefficients or distribution ratios > 1. BAT-calculated metrics for air-breathing organisms are available for organic chemicals with log K_{OW} > 2 and log K_{OA} > 5. Zwitterionic chemicals and quaternary ammonium compounds ("permanently charged") as well as metals and inorganics are currently considered outside the BAT-calculated ("in silico output") AD.

Overview of the BAT Workflow

A conceptual representation of the BAT workflow is presented in Figure 3. The user is required to provide information in four general stages, i) Initialization, ii) Physical-Chemical Properties, iii) Biotransformation, and iv) Bioaccumulation (in vivo, in vitro, in silico). Basically, after establishing the details of the assessment criteria ("B" metric specific classification values), the user has to obtain chemical-specific property information (namely, physical-chemical properties and k_B) and other lines of existing information relevant for "B" assessment (e.g., measured in vivo BCF, QSAR predicted BCF). The user must summarize relevant aspects of the various lines of evidence (LoE) to determine data reliability scores for each LoE. The BAT guides the user through data reliability evaluations with the use of Data Evaluation Templates (DETs) that have been developed for each LoE. The DETs have been developed from standard guidance when available (e.g., OECD 305 for lab BCFs and BMFs and OECD QSAR guidance for QSAR predictions, etc.) and from professional judgement when standard guidance is not currently available (e.g., field metrics).

Figure 3. Conceptual representation of the BAT workflow



The data types (in vivo, in vitro, in silico) and bioaccumulation and TK parameters and metrics handled by the current version of the BAT are summarized for aquatic and terrestrial organisms in Table 1 and Table 2, respectively.

The final stage of the BAT workflow is Integration/Evaluation. This process is automated and takes the user to the **Results** sheet in the software application. The **Results** sheet includes a summary of the user-entered data and associated data reliability scores, output from the in silico bioaccumulation assessment conducted by the BAT, comparisons to benchmark chemicals and other information regarding the overall analysis. The requirements to complete each stage of the BAT are explained in the following sections below whereas technical details of the BAT are provided in the **Appendices**.

Table 1. Lines of Evidence (LoE) and data currently considered for aquatic organisms

Ecosystem	Data Type	Parameter/Metric
Aquatic	In vivo	Biotransformation rate constant Laboratory BCF (steady-state or kinetic) Laboratory BMF (steady-state or kinetic) Field BAF Field BMF Field TMF
	In vitro	Biotransformation rate constant
	In silico	Biotransformation rate constant Laboratory BCF Laboratory BMF Field BAF Field BMF

Table 2. Lines of Evidence (LoE) and data currently considered for homeotherms

Ecosystem	Data Type	Parameter/Metric
Homeotherms	In vivo	Biotransformation rate constant Field BMF Field TMF
	In vitro	Biotransformation rate constant
	In silico	Biotransformation rate constant Laboratory BMF (based on empirical rodent TK data) Field BMF

Initializing the BAT, Relevance Weighting and Threshold Values

The first task of the user is to initialize the BAT with identifying information about the chemical. The user is also required to provide a relevance weighting for each possible "B" assessment metric, which then can be used for ranking in the Weight of Evidence (WoE).

Initialization Form

The Initialization Form is shown as Figure 4. The user is asked to provide information on chemical identity (Name, CAS RN, SMILES code) and whether the chemical under evaluation is a neutral organic chemical or an ionizable organic chemical (IOC). This information is automatically transferred to the various Data Evaluation Templates (DET) used to inform the bioaccumulation assessment.

Relevance Weighting

There is no consensus in the scientific and regulatory communities for relevance weighting for the various bioaccumulation metrics that can be used for a weight of evidence approach for bioaccumulation assessment. Hence, the user is required to indicate their subjective relevance weighting for each "B" assessment metric. The BAT currently considers BCF, BMF, BAF, TMF metrics from various sources

(laboratory in vivo, field in vivo or a combination of in vitro and in silico methods). A score of five indicates maximum relevance whereas a score of zero indicates no relevance.

Threshold Values for Bioaccumulation Categorization

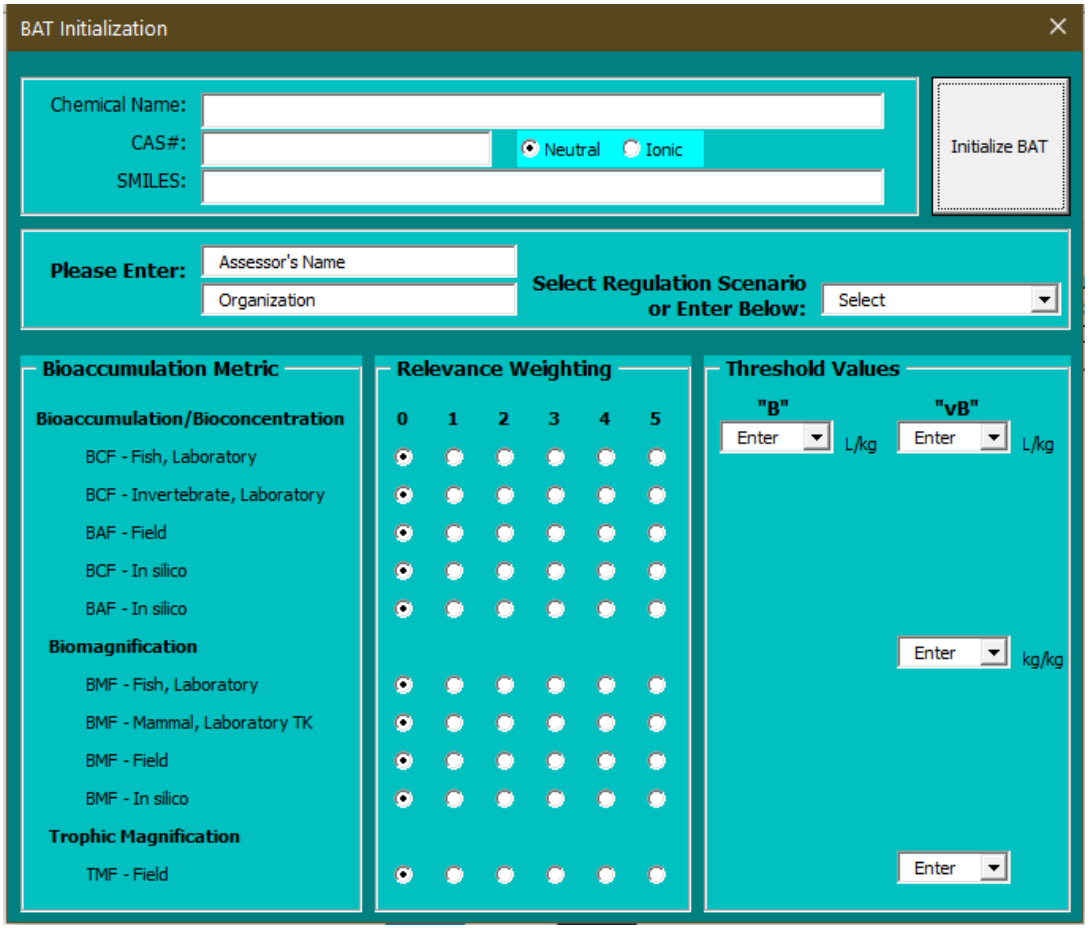
The user is then required to define the threshold values for "B" (bioaccumulative) and "vB" (very bioaccumulative) for the bioaccumulation and bioconcentration metrics, biomagnification metric and trophic magnification metric. Default threshold values under different regulatory schemes (e.g., REACH, CEPA, TSCA) are provided by the BAT and can be selected by the user, but it is the user's responsibility to ensure consistency with regulations and the context-specific objectives of the assessment.

When the "*Initialize BAT*" button is pressed, a Save As dialogue box opens where the user is asked to save the file before proceeding with the assessment. The default settings for saving the file are as shown below:

File name:	BAT (user-entered CAS)
Save as type:	Excel Binary Workbook (*.xlsb)

The user is free to change the file name (and location on the computer) but it is mandatory to save the file as an Excel Binary Workbook or the original BAT workbook (Ver.2.02) will be corrupt and unable to run additional assessments. Once saved, the user is taken to the **BAT Main** interface which serves to guide the user through the various data entry and data evaluation procedures.

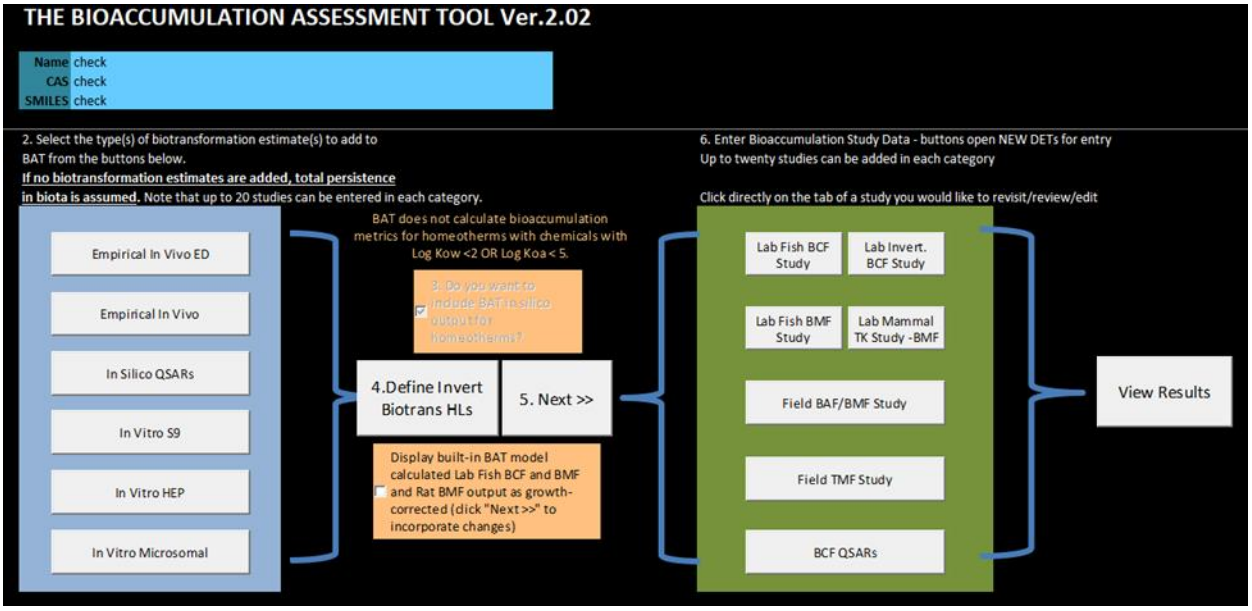
Figure 4. BAT Initialization form



The BAT Main User Interface

A screen capture of the key elements of the **BAT Main** interface sheet is presented in Figure 5. The user is first required to enter chemical property data ("*Physical-Chemical Properties*" button) before other buttons on the sheet become active. The user can then enter data on biotransformation (empirical in vivo, in silico (QSARs), in vitro S9, in vitro HEP, in vitro microsomal) or click on the "*Next*" button to activate the buttons for bioaccumulation data entry (laboratory BCF for fish and invertebrates, laboratory BMF for fish, laboratory TK data for rodents, field BAF/BMF, field TMF, BCF QSARs). Once the user has completed data entry and evaluation, the final summary results are tabulated by clicking on the "*View Final Results*" button. The user can also view interim results (i.e., before completion of data entry and evaluation) by clicking on the "*View Interim Results*" button.

Figure 5. Screen capture of the key elements of the BAT Main interface



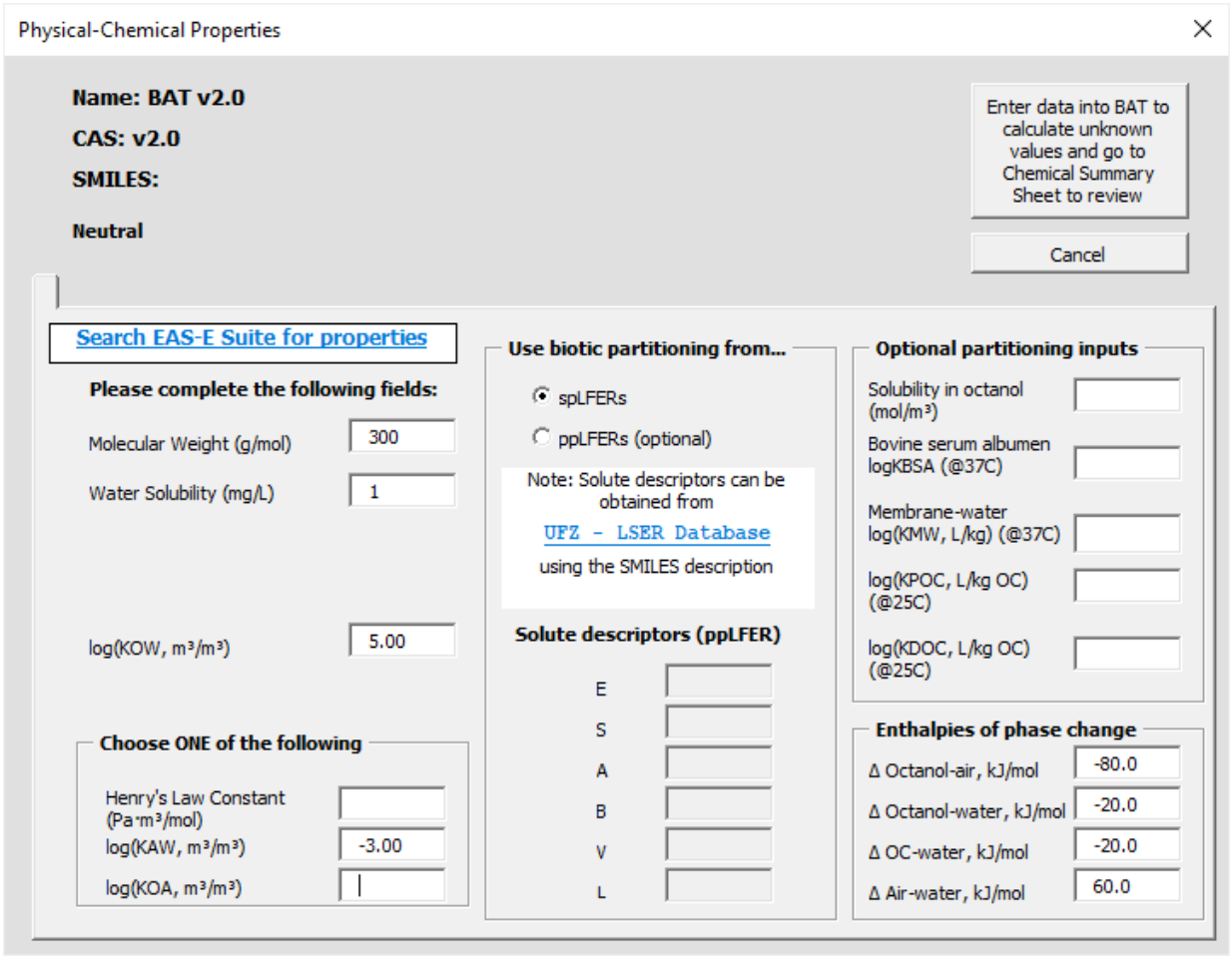
Physical-Chemical Properties—Neutral Organic Chemical

Basic Property Information

Basic property information is entered on a user form and then compiled and presented on the **Chemical Summary** worksheet. A screen capture of the property input user form for neutral organic chemicals is shown in Figure 6; the **Chemical Summary** worksheet is shown in Figure 7.

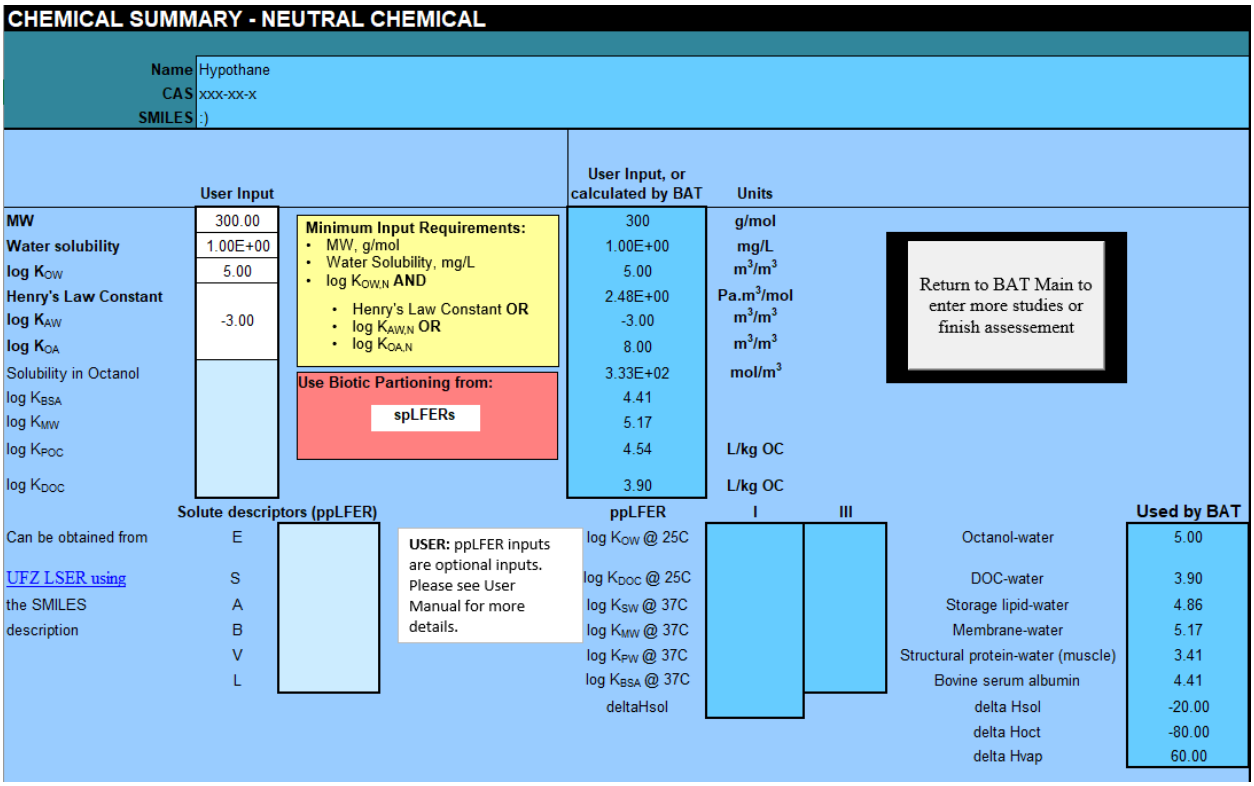
The mandatory user inputs for the properties of the neutral organic chemical under evaluation include: i) molecular weight (MW, g/mol), water solubility (mg/L), and log K_{OW} and ii) Henry's Law Constant (H, Pa m^3/mol) or log K_{AW} (dimensionless) or log K_{OA}. The property data can be measured (preferred) or predicted values from property estimation software such as EPI Suite [48] and other sources (e.g., US EPA Chemistry Dashboard: https://comptox.epa.gov/dashboard/ and EAS-E Suite: www.eas-e-suite.com). The user is reminded to be careful that the values entered correspond with the units required by the BAT. If available, it is also possible to enter data on solubility in octanol (mol/m^3) and partitioning to bovine serum albumin, phospholipids (membranes), dissolved organic carbon and particulate organic carbon (log K_{BSA}, log K_{MW}, log K_{DOC} and log K_{POC} respectively).

Figure 6. User-form for entering property values for neutral organic chemicals



The user can choose how the BAT calculates key environmental and biological partition coefficients by selecting to use either single parameter linear free energy relationships (spLFERs) or polyparameter linear free energy relationships (ppLFERs). As a default, the BAT applies spLFERs to estimate the partitioning properties not provided by the user. If ppLFERs are selected and the required solute descriptors are provided by the user, the BAT will calculate environmental and biological partition coefficients using this approach, as documented in the Appendices (A2. Physical-Chemical Properties). New in Ver.2.0 is the input of the enthalpies of phase change in which allows for the temperature-correction of abiotic and biotic partition coefficients (e.g., from 25 ºC to an ambient environmental temperature of 10 ºC).

Figure 7. Chemical summary display sheet for neutral organic chemicals



Partitioning Property Estimates (spLFER)

Single parameter linear free energy relationships (spLFERs) typically have the following form:

$$logK_{ij} = a \log K_{xy} + b$$

where log K_{ij} is the property value to be predicted, log K_{xy} is the known property value and *a* and *b* are regression coefficients. Clicking the *"Enter Data into BAT…"* button on the user-entry form (Figure 6) causes the BAT to automatically generate and display predicted partition coefficients for log K_{POC}, log K_{DOC}, storage lipid-water partitioning (log K_{SW}), membrane-water partitioning (log K_{MW}), structural protein-water partitioning (log K_{PW}) and bovine serum albumin-water partition (log K_{BSA}) in the "User Input, or calculated from spLFER" section of the input sheet (Column E, see also Figure 7). If selected, the biotic partition coefficients based on spLFERs are used to estimate the overall sorption capacity of the organisms for the in silico bioaccumulation assessment conducted by the BAT and elsewhere (e.g., as part of the in vitro-in vivo extrapolation). The various spLFERs utilized by the BAT are documented in the Appendices (A2. Physical-Chemical Properties). The user-entered or spLFER-calculated values used by the model are displayed in the "Used by BAT" box (see Figure 7).

Solute Descriptors and Partitioning Property Estimates (ppLFER)

As noted above, the user is given the option of using spLFERs or ppLFERs to estimate the additional partitioning property estimates required by the BAT.

The general forms of ppLFER equations are as follows:

$$logK_{ij} = sS + aA + bB + vV + lL + c$$

$$logK_{ij} = eE + sS + aA + bB + vV + c$$

where log K_{ij} is the property value to be predicted, E, S, A, B, V and L are chemical-specific solute descriptors and e, s, a, b, v, l and c are regression coefficients.

To operationalize the ppLFER option, the user must select the ppLFER option and enter solute descriptors into the user-entry form (Figure 6) which are then used to calculate the various partition coefficients displayed in the sheet. A link to the UFZ LSER Database is provided to assist the user in obtaining the required inputs. The required solute descriptors (E, S, A, B, V, L) represent the ability of the chemical to engage in the various types of molecular interactions (e.g., hydrogen bonding – A, B, van der waals – V, L) governing the relative affinity of the phases for the chemical [49]. Two ppLFER equations are included for K_{OW}, K_{DOC}, K_{SW}, K_{MW}, K_{PW} and K_{BSA} which are automatically calculated when the user hits the "*Assess Input and Reliability"* button. These equations are documented in the Appendices (A2. Physical-Chemical Properties).

If the user enters the required solute descriptors and selects ppLFER, the overall chemical sorption (storage) capacity of the organism for the in silico bioaccumulation assessment conducted by the BAT is estimated using the (average) partition coefficients generated using these equations rather than the spLFER-based values. The ppLFER-based values used by the model are displayed in the "Used by BAT" box (see Figure 7). Log K_{OW} becomes an optional input if ppLFERs are selected; however, a user entered K_{OW} will be used preferentially over K_{OW}s calculated by the ppLFER.

Biotransformation Rate Constant Summary

Biotransformation rate constant or half-life data (in vivo, in vitro, in silico) are processed by the BAT as part of the second stage of the assessment (see Biotransformation Data section). For convenience and transparency, all the processed and quality-assessed data are automatically summarized on the **Chemical Summary** sheet after it is entered by the user and **no data on biotransformation can/should be entered here** during the first stage of data collection.

NOTE: For modeling purposes (see BAT in silico bioaccumulation assessment), the reliability score-weighted mean of the standardized biotransformation rate constants is used for all calculations. Uncertainty in the standardized biotransformation rate constants is reflected in the confidence factor (CF) and is propagated into the BAT calculations resulting in a lower range and upper range estimate reported in addition to the estimate corresponding to the reliability score-weighted mean.

Physical-Chemical Properties—Ionizable Organic Chemical

Basic Property Information

Basic property information for IOCs is also entered on a user form and then compiled and presented on a **Chemical Summary** worksheet. Screen captures of the property input user forms for ionizable organic chemicals is shown in Figure 8 and Figure 9 and the **Chemical Summary** worksheet is shown in Figure 10.

Note that the data input sheet for the physical-chemical properties of ionizable organic chemicals (IOCs) is substantially more involved than the data input sheet for the physical-chemical properties of neutral organic chemicals. The additional complexity stems from the fact that IOCs can exist in neutral and charged form in the environment, as determined by the pH of the system and the dissociation constant(s) of the IOC (pKa). There is one section of the user form for the properties of the neutral form of the chemical (partition coefficients) and another section for the combined properties of the neutral and charged form (i.e., distribution ratios such as D_{OW}).

Figure 8. User form for entering IOC type, pKa and properties of the neutral form of an IOC

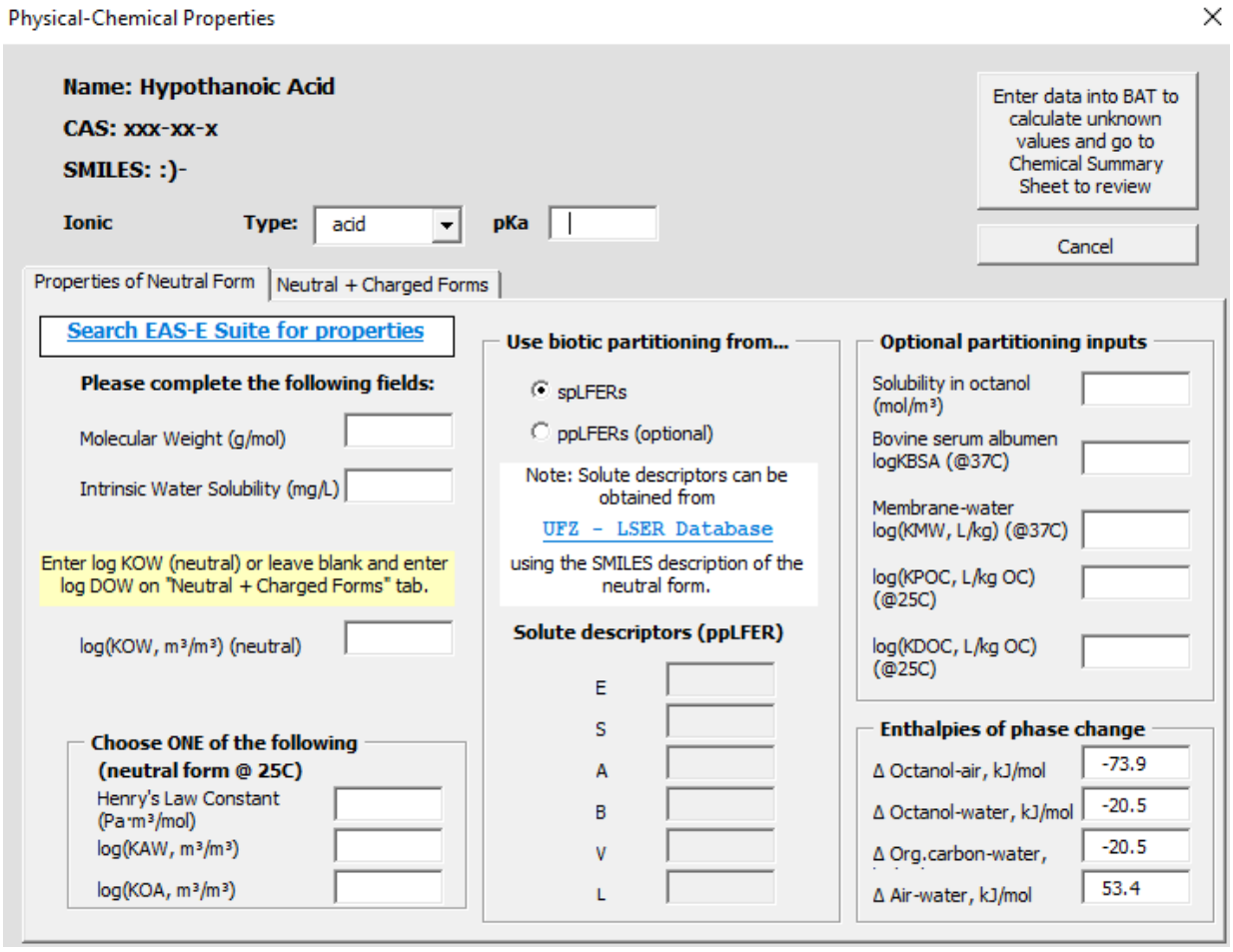
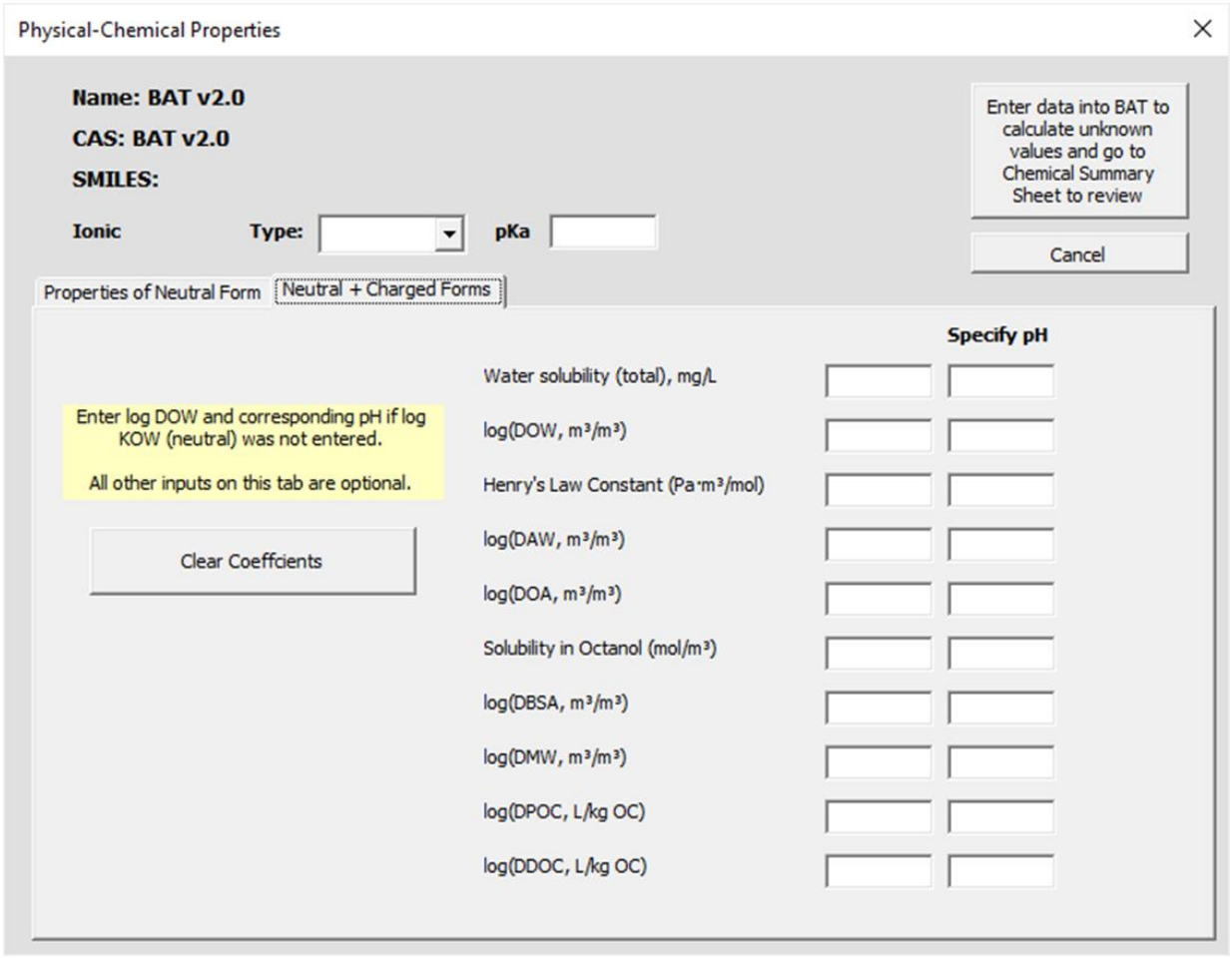


Figure 9. User form for entering distribution ratios for IOCs

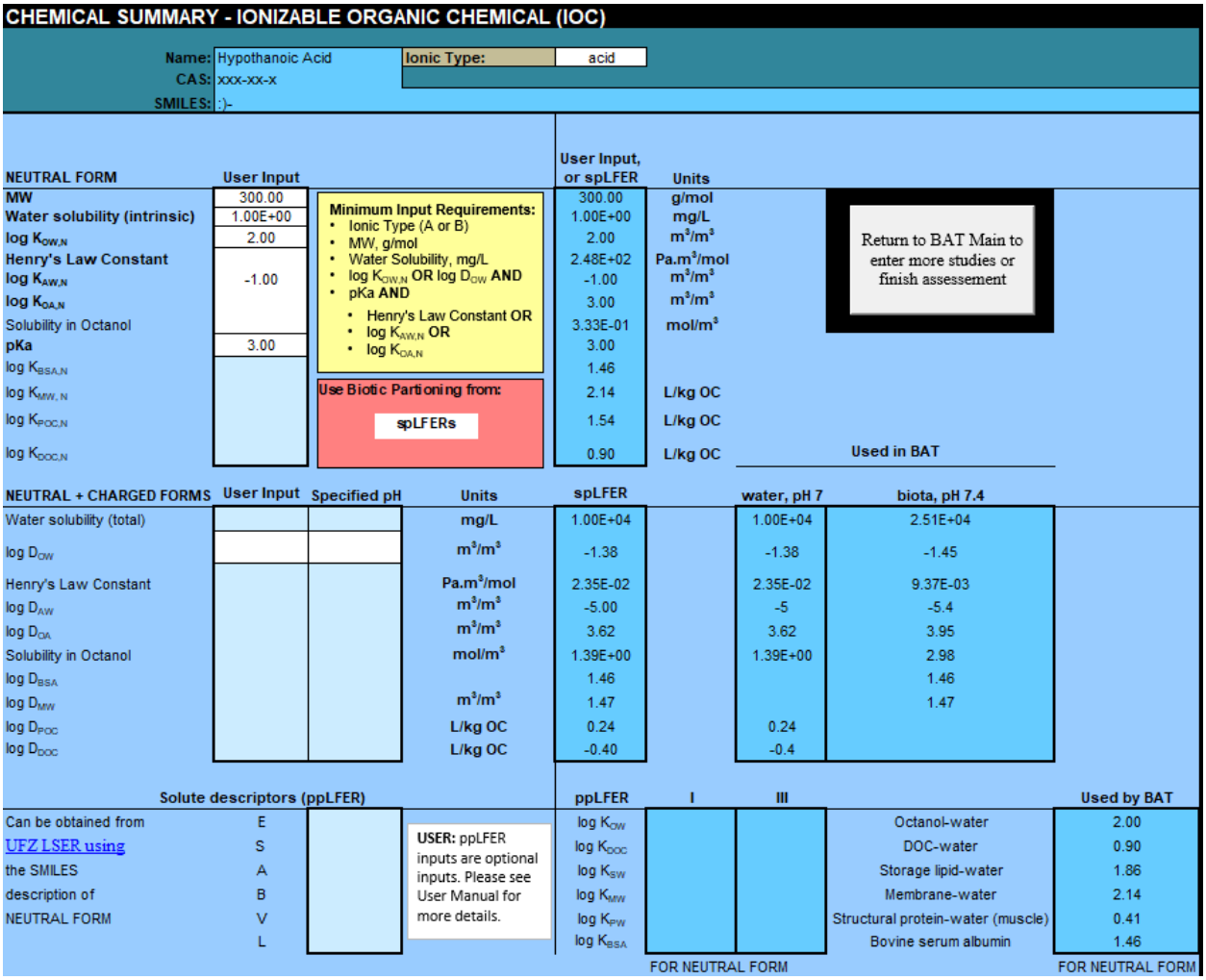


The user is first required to specify the type (acidic, basic) and a dissociation constant (pKa) for the IOC of interest. The current version of the BAT is only able to handle a single pKa value and therefore the user is expected to enter the pKa of the strongest acidic or basic functional group on the molecule. Multi-protic chemicals should be evaluated with appropriate awareness of the challenges of evaluating these chemicals due to substantial data gaps in "B" data for these chemicals (i.e., additional uncertainty).

The mandatory property information required for ***the neutral form*** of an ionizable organic chemical (Figure 8) is molecular weight and intrinsic water solubility (S_0). The user has the option to enter the octanol-water partition coefficient of the neutral form ($K_{OW,N}$) or to address this property value through a distribution ratio (Figure 9). The user is then required to enter one of the following properties of the neutral form; Henry's Law Constant (H, Pa m^3/mol), log $K_{AW,N}$ (dimensionless) or log $K_{OA,N}$.

After entering property data for the neutral form of the chemical (Figure 8), the user can enter available empirical distribution ratios (e.g., D_{OW} and D_{AW}) in the appropriate section of the user form (Figure 9). It is critical for the user to enter the measured distribution ratio and the pH of the system the measurement was taken in. If no pH is entered by the user, the user-defined distribution ratio will be ignored by the BAT and one will be calculated internally. All user-entered and BAT-calculated data are compiled and summarized by clicking on the "*Enter Data into BAT….*" button, which takes the user to the **Chemical Summary** worksheet for IOCs (Figure 10).

Figure 10. Chemical summary sheet for ionizable organic chemicals (IOCs)



Partitioning Property Estimates (spLFER)

As for neutral organic chemicals, property predictions (spLFERs) are automatically conducted by the BAT if this option is selected and the "*Assess Input and Reliability"* button is pressed. These procedures are applied only to the property data of the neutral form (i.e., partition coefficients).

Solute Descriptors and Partitioning Property Estimates (ppLFER)

Partitioning property data for the neutral form of IOCs can be generated using solute descriptors entered by the user and the same ppLFERs applied to neutral organic chemicals (see above).

Scaling Factors (SF) for IOCs

In the absence of user-entered distribution ratios, the BAT estimates the partitioning behavior of the charged form of the chemical using scaling factors [31, 50] as

$$K_{OW,I} = \rho K_{OW,N}$$

where $K_{OW,I}$ is the (apparent) octanol-water partition coefficient of the charged form of the chemical, $K_{OW,N}$ is the octanol-water partition coefficient of the neutral form of the chemical and ρ is the scaling factor. Default assumptions for relevant scaling factors are presented in the Appendices (A2. Physical-Chemical Properties). Distribution ratios are calculated by the BAT and displayed in the pH-specific partition coefficient section of the **Chemical Summary** sheet (Figure 11).

Figure 11. pH-specific distribution ratios calculated by the BAT

	pH-specific partition coefficients						
	Air, pH 5	Water, pH 7	Soil, pH 6	Sed pH 7	ioPC,various	Org pH 7.4	pH ioPC
Water solubility (total)	3.99E+02	1.00E+04	1.00E+03	1.00E+04		2.51E+04	
log DOW	-5.50E-01	-1.38E+00	-8.80E-01	-1.38E+00		-1.45E+00	
Henry's Law Constant	5.90E-01	2.35E-02	2.35E-01	2.35E-02		9.37E-03	
log DAW	-3.60E+00	-5.00E+00	-4.00E+00	-5.00E+00		-5.40E+00	
log DOA	3.05E+00	3.62E+00	3.12E+00	3.62E+00		3.95E+00	
Solubility in Octanol	3.75E-01	1.39E+00	4.39E-01	1.39E+00		2.98E+00	
log DBSA	1.46E+00	1.46E+00	1.46E+00	1.46E+00		1.46E+00	
log DMW	1.48E+00	1.47E+00	1.47E+00	1.47E+00		1.47E+00	
log DPOC		2.40E-01	2.50E-01	2.40E-01			

Biotransformation Rate Constant Summary

Biotransformation rate constant or half-life data (in vivo, in vitro, in silico) are processed by the BAT as part of the second stage of the assessment (see Biotransformation Data section). For convenience and transparency, the processed and evaluated biotransformation data are automatically summarized on the **Chemical Summary** sheet after it is entered by the user. **No data should be entered here during the first stage of the assessment**.

NOTE: For modeling purposes (see BAT in silico bioaccumulation assessment), the reliability score-weighted mean of the standardized biotransformation rate constants is used for all calculations. Uncertainty in the standardized biotransformation rate constants is reflected in the confidence factor (CF) and is propagated into the BAT calculations resulting in a lower range and upper range estimate reported in addition to the estimate corresponding to the reliability score-weighted mean.

Biotransformation Rate and Half-life Data

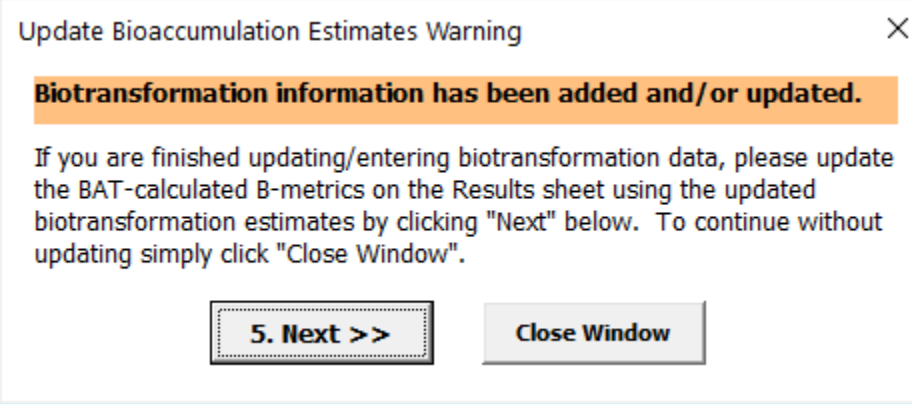
Biotransformation is a key process mitigating the bioaccumulation potential of hydrophobic organic chemicals in aquatic and terrestrial organisms [12, 18-20]. It is a critical consideration for refining the bioaccumulation assessment that would otherwise be based only on partitioning data (e.g., log K_{OW}) and is

therefore a vital component of the overall B assessment. An in vivo k_B database [18] and several QSARs for predicting k_B and biotransformation half-lives (HL_B) for fish [40-42, 48] exist. The QSARs have been developed and evaluated following Organization for Economic Cooperation and Development (OECD) QSAR guidance [44, 45]. In vivo databases and QSARs [39, 43] for predicting total elimination half-lives (HL_T) and HL_B have also been developed for humans following OECD QSAR guidance [44, 45]. Methods for estimating in vitro metabolism (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 [51, 52]. QSARs for predicting clearance rates from in vitro data streams also exist, e.g., [53, 54]. Standardized in vitro biotransformation rate methods for fish S9 [55] and hepatocyte [56] assays have evolved. Methods for extrapolating the in vitro rates to in vivo rates have also evolved in human health, e.g., [57, 58] and ecological sciences, e.g., [59, 60].

The **BAT Main** interface (Figure 5) allows the user to include several LoE for biotransformation rates including i) empirical in vivo data, ii) in silico data (i.e., QSAR predictions), and iii) in vitro data (S9, hepatocyte, microsomal). The procedures for handling these data are described in the following sections. There are two basic stages to complete, i) key test details and quantitative information and ii) the data quality (**reliability**) assessment. If no data are available or the user chooses to omit such data, the user can click on the "*Next*" button to activate the Bioaccumulation Data entry buttons. In this case, the chemical is assumed to have "Total Persistence" (see below).

NOTE: If biotransformation studies are updated or added AFTER bioaccumulation estimates have been made, the user will be prompted to update the bioaccumulation estimates already entered, or predicted by, the BAT. This prompt (Update Bioaccumulation Estimates Warning) is shown in Figure 12.

Figure 12. Update bioaccumulation estimates warning



Total Persistence

Under the "Total Persistence" scenario, the chemical under evaluation is assumed to be stable (i.e., not subject to biotransformation). Bioaccumulation is then simply a function of the partitioning (distribution) properties of the chemical and the biological properties of the organism. It is generally expected that at least some information on biotransformation will be available (e.g., in silico estimates). Nevertheless, simulations with assumed total persistence can serve to quantitatively indicate the relative importance of biotransformation in determining the bioaccumulation potential of the chemical.

Dietary Absorption Efficiency, E_D

The chemical dietary absorption efficiency (E_D) quantifies the fraction of chemical absorbed from the gut into the gastrointestinal tissue (GIT) following ingestion [1]. Values of E_D vary from chemical to chemical and organism to organism and can be estimated using models. The B and TK models in BAT Ver.1.0 did not consider the possibility for chemical degradation in the GIT. The B models in BAT Ver.2.0 for vertebrates (fish and mammals) now incorporate the mechanistic E_D model described in [1] while the E_D model for invertebrates is based on the model described in [2]. BAT Ver.2.0 the user now has the option to enter empirical E_D values to replace the default model E_D calculations for vertebrate species (fish and mammals). In BAT Ver.2.0 there is a new E_D input sheet to record study information (Figure 13) and an associated DET to assess the reliability of this information (Figure 14). If there are multiple entries for each organism type, the entered values are averaged, and the confidence interval is estimated for each organism type. The average empirical E_D value is used in the models and the confidence interval is used to assess the level of uncertainty in the BAT model estimates.

Key Details and Quantitative Information

The input sheet for *in vivo* dietary absorption efficiency study entry and assessment is shown in Figure 13 and Figure 14. Up to 20 E_D values can be entered and assessed. Mandatory inputs include definition of organism type "fish" or for mammals, "herbivore" or "carnivore". Entry of "carnivore" would be appropriate for omnivorous mammals as well. The source/citation, species assessed and the dietary efficiency value, entered in % as "85" rather than 0.85 for example. Following this, the DET (Figure 14) must be filled out using a "Y" or "N" in each of the first two columns (entering "N" results in an automatic "FAIL" and the value in that row will not be used. The user would then enter "Y" in only the most applicable of columns 4 through 7. If there is an issue with the study that renders it unacceptable, then the user may indicate so by entered "TRUE" in column 8 and provide text for a justification in column 9.

Figure 13. Chemical dietary absorption efficiency study input sheet

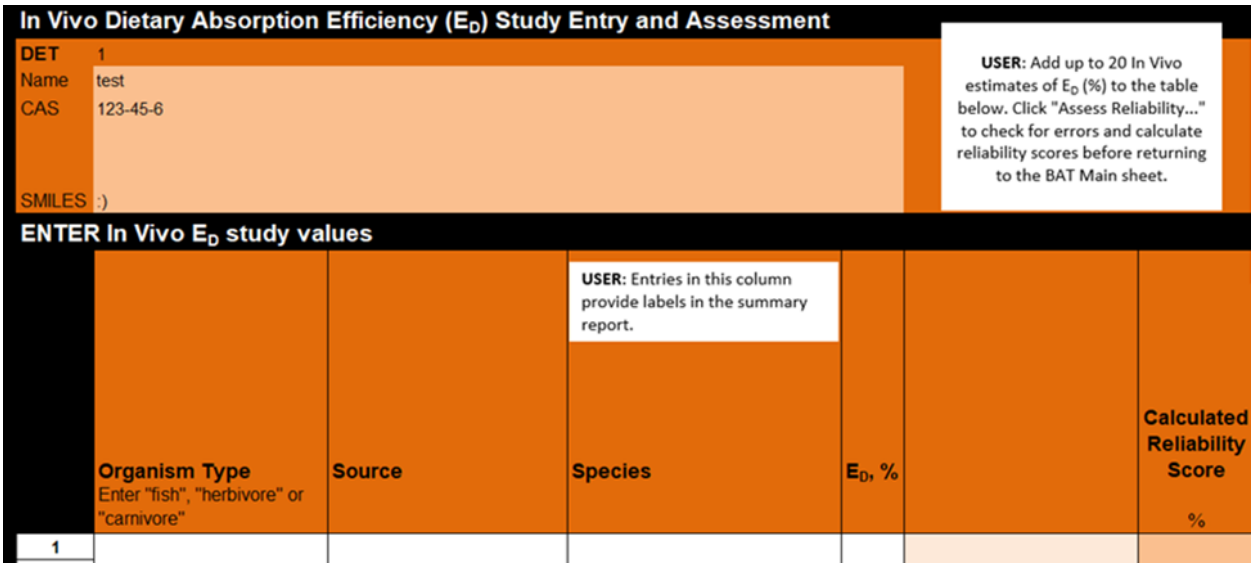


Figure 14. Chemical dietary absorption efficiency study DET

Data Evaluation Template (DET)

Reliability criteria for evaluating data reliability of a fish or mammalian chemical dietary uptake efficiency (E_D)

1	2	3	4	5	6	7	8	9
Is the species known? (**Enter Y or N**)	Is the value of ED less than or equal to 100%? (**Enter Y or N**)		The dietary absorption efficiency E_D measured directly in vivo.	The dietary absorption efficiency E_D was estimated using a toxicokinetic model (with high confidence).	The dietary absorption efficiency E_D was estimated using a toxicokinetic model (with moderate confidence).	Thedietary absorption efficiency E_D was estimated using a toxicokinetic model (with low confidence).	**CRITICAL FAIL (Overall Reliability Score = 0)**	**Justification for CRITICAL FAIL required**
			Please choose the statement that best describes the study by entering a "Y" in the appropriate column.				**Enter FAIL or leave blank**	

Empirical In Vivo Biotransformation

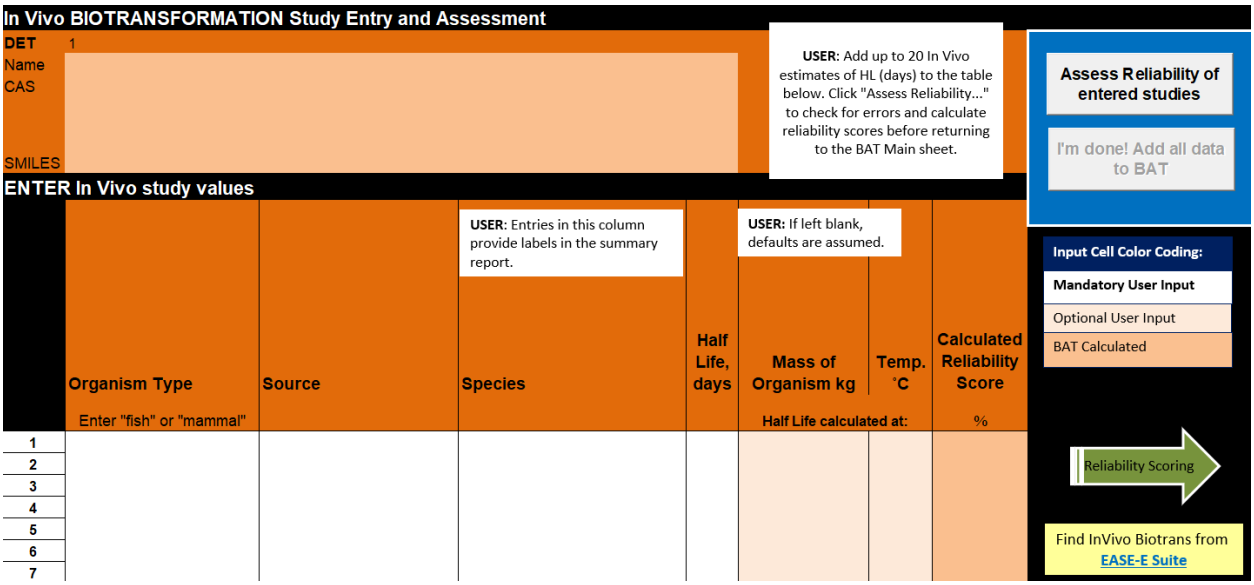
Arnot et al. collected and critically evaluated bioaccumulation data for fish and subsequently derived in vivo biotransformation rate constants (k_B, 1/d) using a toxicokinetic model for 702 chemicals [17, 18]. Arnot et al. [39] also published a database of biotransformation half-life data in humans. In some cases, it may also be justifiable to use estimated in vivo biotransformation rate constants for similar chemicals, based on read-across. A similarity scoring tool and guidance for this process is not established nor implemented in the current version of the BAT.

Any use of surrogate information requires clear documentation by the user. In vivo biotransformation data obtained by the user can also be included assuming the required information is also available.

Key Details and Quantitative Information

The input sheet for in vivo biotransformation rate and half-life (HL) data is shown in Figure 15. Up to 20 in vivo biotransformation values can be entered. The mandatory user inputs for the in vivo biotransformation sheet data include i) organism type (user should type in fish, mammal), ii) source of the data, iii) species, and iv) the biotransformation half-life in days. Ideally the user will provide the mass of the organism and the temperature that the HL applies to. In the absence of this information, the BAT assumes a mass of 0.01 kg and temperature of 15 °C for fish and a mass of 70 kg and temperature of 37 °C for mammals. However, the reliability score of the estimate is set to zero in such cases ("Critical Fail"; see Table 3). If the user happens to be applying the BAT to a chemical included in the published databases [39], the reported biotransformation half-life can be used directly.

Figure 15. Input sheet for in vivo biotransformation data



When compiled and displayed on the **Chemical Summary** sheet, all in vivo HL_B are converted to rate constants assuming first-order kinetics (i.e., $k_B = \ln(2)/HL_B$) and then normalized to a 0.01 kg organism at 15 °C for fish data and to a 70 kg organism at 37 °C for mammalian data as:

$$k_{B,N} = k_{B,i}(W_N/W_i)^{-0.25}\exp\left[0.01(T_N - T_i)\right]$$

where $k_{B,N}$ is the normalized biotransformation rate constant, $k_{B,i}$ is the biotransformation rate constant entered by the user for a given (Q)SAR, W_N is 0.01 kg (or 70 kg), W_i is the mass of the organism entered by the user (kg), T_N is 15 °C (or 37 °C) and T_i is the temperature entered by the user. For the simulations conducted by the BAT at later stages of the application (e.g., calculated laboratory BCF, laboratory BMF, field BAF, field BMF), $k_{B,N}$ is re-scaled to match the size of each organism and the relevant temperature.

Data Reliability Assessment (Data Evaluation Template)

The data quality considerations currently incorporated in the DET for in vivo biotransformation data are presented in Table 3. Three of the considerations are Pass/Fail and the remaining considerations are direct scores (%) that are assigned to each user-entered half-life estimate depending on which one is fulfilled.

Table 3. Data quality considerations for in vivo biotransformation rate constant estimates

#	Quality Criterion/Consideration	Maximum Score
1	Is the species known?	Pass/Fail
2	Is the body mass of the test organism known or assumed with reasonable confidence?	Pass/Fail
3	Is the body temperature known or assumed with reasonable confidence?	Pass/Fail
4	Is the biotransformation half-life measured directly in vivo?	95%
5	Is the biotransformation half-life estimated using a toxicokinetic model (with high confidence)?	80%
6	Is the biotransformation half-life estimated using a toxicokinetic model (with high confidence)?	65%
7	Is the biotransformation half-life estimated using a toxicokinetic model (with high confidence)?	50%
8	Critical Fail for other reason (override; quality score = 0). The user should provide a brief statement in the space provided justifying this decision.	Fail

Note that Q4 – Q7 are mutually exclusive and therefore only one option should be selected by the user. In the current version of the BAT, the DET for in in vivo biotransformation data must be completed manually be the user. Scroll to the right of the worksheet and enter Y or N as appropriate for each question.

In Silico Biotransformation Rate and Half-life Data

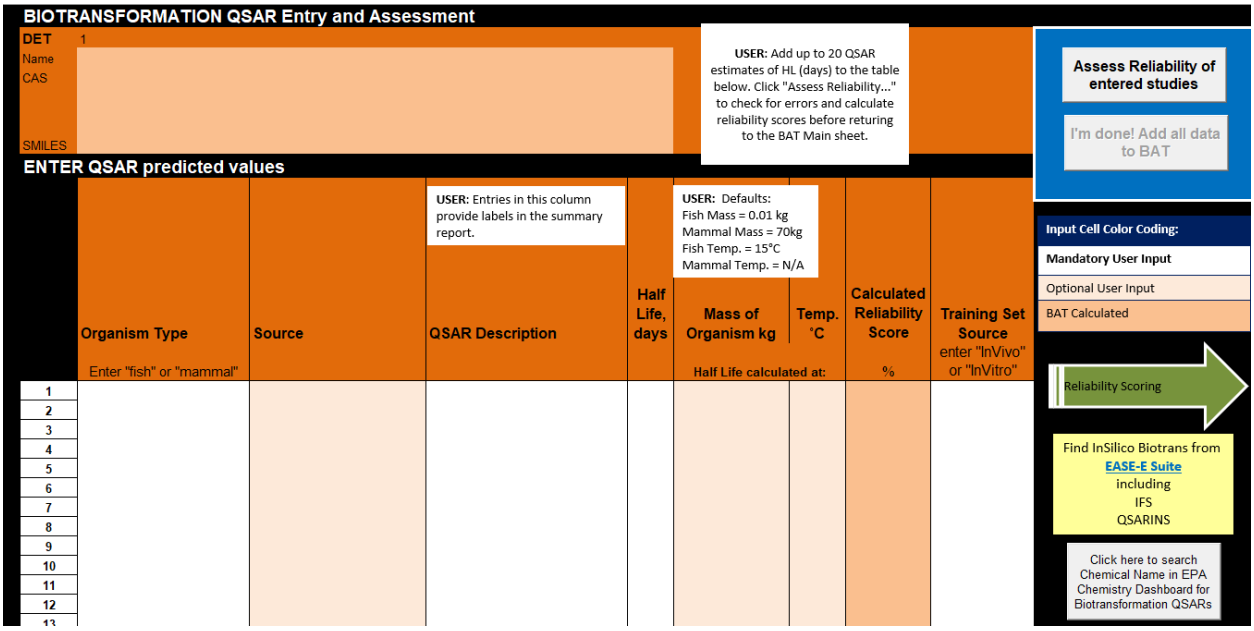
There are a few approaches for predicting biotransformation half-lives for organic chemicals in the literature. For example, Arnot et al. published a database of biotransformation rate constants (k_B, 1/d) for organic chemicals in fish [17, 18] which was subsequently used to derive a HL_B-QSAR [40]. This QSAR is publicly available as part of the BCFBAF v3.01 module in EPI Suite v4.11. Output from EPI Suite is presented as log HL_B, HL_B and k_B. Some care is required when selecting the relevant EPI Suite output (i.e., HL_B) as input for the BAT. Other options for estimating biotransformation rate constants in fish include Brown et al. [41] (available in www.eas-e-suite.com), the QSARs implemented in the EPA Chemistry Dashboard, (i.e., OPERA QSARs https://comptox.epa.gov/dashboard/) and those developed by researchers at Insubria (QSARINS, http://www.qsar.it/, e.g., [42]). Links to these QSARs are provided on the **QSAR BioTrans** sheet.

The user can access in silico biotransformation estimates using EAS-E Suite
www.eas-e-suite.com

Key Details and Quantitative Information

The input sheet for in silico biotransformation rate and half-life data is shown in Figure 16. The mandatory user inputs for in silico biotransformation data include, i) organism type (user should type in fish, mammal), ii) (Q)SAR description, iii) the predicted biotransformation half-life (HL) in days and the iv) the type of training set data (in vitro or in vivo estimates) used to develop the QSAR. Ideally the user will be able to provide the mass of the organism and the temperature that the HL applies to. For example, the BCFBAF v3.01 module in EPI Suite v4.11 generates predicted half-lives for a 0.01 kg fish at 15 °C and these normalized values should be selected for input into the BAT. If the user does not enter data in these two columns for fish, the mass and temperature will be assumed to be the same as the BCFBAF v3.01 module.

Figure 16. Input sheet for in silico biotransformation rate and half-life data



The BAT currently allows the user to enter up to 20 in silico biotransformation half-life estimates, which are assessed and then reported on the **Chemical Summary** sheet (Biotransformation summary). Additional details are given below.

As with in vivo data, all in silico biotransformation half-lives entered by the user are converted to rate constants ($k_B = \ln(2)/HL_B$) and then normalized to a 0.01 kg organism at 15 °C for fish data and to a 70 kg organism at 37 °C for mammalian data. See the in vivo biotransformation section for the equation used.

Data Reliability Assessment (Data Evaluation Template)

Table 4 summarizes the data quality considerations currently incorporated in the DET for in silico biotransformation data based on OECD principles for the validation of (Q)SARs for regulatory purposes (http://www.oecd.org/env/ehs/risk-assessment/validationofqsarmodels.htm). Quality criteria considered critical are indicated by Pass/Fail. If any one of these criteria are not met, the (Q)SAR is considered unreliable and the predicted HL_B is given a reliability score of zero. In the current version of the BAT, the DET for in silico biotransformation data must be completed manually be the user. Scroll to the right of the worksheet and enter Y or N as appropriate for each question.

Table 4. Data quality considerations for in silico biotransformation rate constant estimates

#	Quality Criterion/Consideration	Maximum Score
1	Is a defined endpoint clearly presented?	Pass/Fail
2	Is the (Q)SAR expressed in the form of a transparent and unambiguous algorithm?	Pass/Fail
3	Is the (Q)SAR associated with appropriate measures of goodness-of-fit, robustness and predictivity?	Pass/Fail
4	Is the (Q)SAR associated with a defined domain of applicability?	15
5	Does the (Q)SAR provide a mechanistic interpretation for the estimate?	15
6	Is the prediction within the stated applicability domain of the QSAR?	30
7	Was the (internal validation) $r^2 > 0.7$?	15
8	Was the (external validation) $q^2_{ext} > 0.5$?	15
9	Critical Fail for other reason (override; quality score = 0). The user should provide a brief statement in the space provided justifying this decision.	Fail

The user is responsible for completing the DET for each QSAR prediction included in the BAT. Data required to complete the DET can often be found in the QSAR-associated QMRF documents or publications.

In Vitro Biotransformation Data—Liver S9, Hepatocytes, Microsomes (Liver)

In vitro biotransformation studies using liver cells (primary or cryopreserved hepatocytes) and subcellular fractions (S9 or microsomes) are becoming increasingly common in ecotoxicology [16, 56, 61, 62]. 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 [26-28, 63].

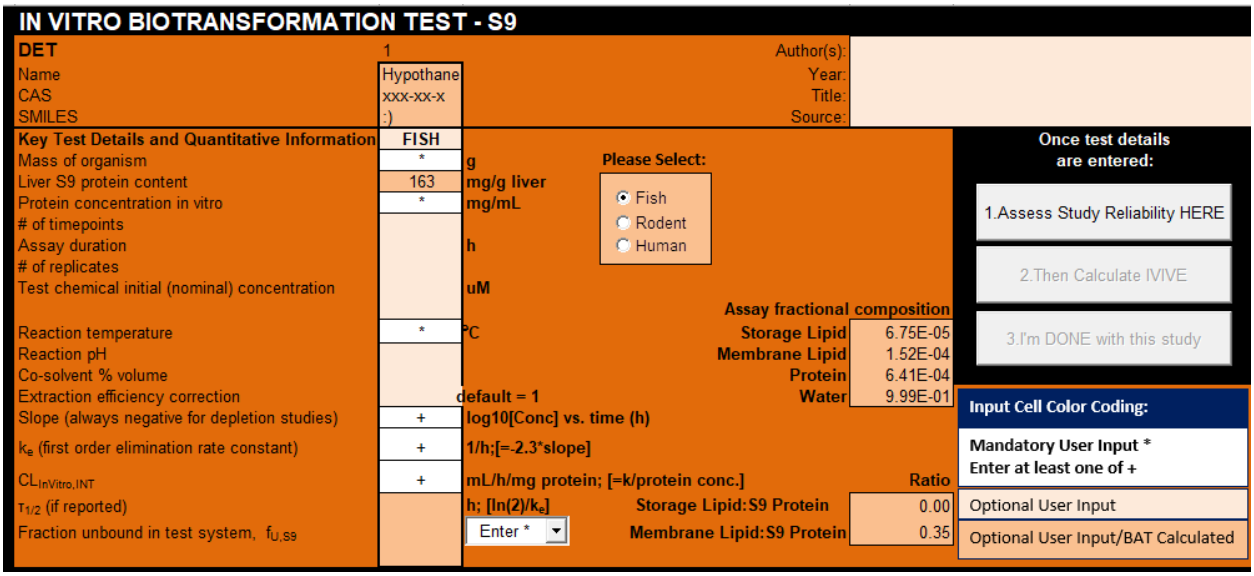
The main objective of these tests is to obtain an estimate of the first-order depletion rate constant of the parent chemical in the test system (k_e). This rate constant can be measured from the loss of the parent chemical (preferable) or inferred from the rate constant for product formation. The in vitro first-order depletion rate constant can subsequently be converted to more biologically-relevant metrics such as intrinsic liver clearance and, through in vitro-in vivo extrapolation (IVIVE), to obtain an estimate of the whole-body biotransformation rate constant (k_B).

Key Test Details and Quantitative Information

The purpose of the key test details and quantitative information section is to collect relevant information for calculating the intrinsic in vitro clearance ($CL_{IN\ VITRO,\ INT}$). Information about the test relevant for the data reliability scoring can also be entered here (e.g., temperature, pH, number of replicates). The key test details and quantitative information sections for liver S9 derived from fish are shown in Figure 17. The inputs for studies using hepatocytes and liver microsomes are similar and screen captures of the entry sheets for such data are not presented in this document.

The user can access in vitro biotransformation estimates from EAS-E Suite
www.eas-e-suite.com

Figure 17. Key test details and quantitative information section of the BAT for liver S9



The mandatory user input includes i) organism type (fish, rodent or human), ii) mass of organism, iii) protein concentration (S9, microsome) or cell concentration (hepatocyte) and iv) at least one of the slope of the depletion curve, k_e, the first order elimination rate constant and/or $CL_{InVitro,Int}$, the intrinsic clearance rate from the in vitro test. Default assumptions for liver S9 protein content, hepatocellularity (10^6 cells / g liver) and microsomal protein content can be opted for by the user and are based on published values, e.g., [58, 61, 64]. See the Appendices (A3. In Vitro Biotransformation Rate Data; A4. In Vitro-In Vivo Extrapolation (IVIVE)) for additional details. It is also necessary to enter a value for the fraction unbound in the test system ($f_{U,S9}$). The default QSAR approaches are taken from the literature [61, 64] but the user can enter their own data if desired. The fractions unbound in the test system can also be calculated using the "compositional approach", whereby $f_{U,assay}$ is a function of assay composition (storage lipid, membrane lipid, protein and water content) and partitioning properties. The protein content of the assay is taken to be equal to the value entered by the user (mg/ml · 1/1000) and the storage lipid and membrane lipid contents estimated using the lipid:protein ratios on the input sheet. The default lipid:protein ratios are based on the available literature [65, 66] but can be changed by the user. The equations implemented in the BAT for the fraction unbound in all test systems are documented in the Appendices (A3. In Vitro Biotransformation Rate Data).

When the "*Assess Study Reliability HERE"* button is clicked, the BAT automatically calculates the intrinsic in vitro clearance ($CL_{IN\ VITRO,\ INT}$) once the DET is completed (see below). In brief, the slope of the depletion curve is used to calculate the first-order elimination rate constant (k_e) and half-life (HL) and the intrinsic in vitro clearance is then a function of k_e and the concentration of liver S9 protein (mg/ml), hepatocytes (10^6 cells/ml) or microsomal protein (mg/ml). See the Appendices (A3. In Vitro Biotransformation Rate Data) for additional details.

Data Reliability Assessment (Data Evaluation Template)

The data quality considerations for an in vitro S9 biotransformation study are based on OECD guidance documents [26-28] and are summarized in Table 5.

Table 5. Data quality considerations for in vitro S9 biotransformation data

#	Quality Criterion/Consideration	Maximum Score
Quality Assurance		
1	Consideration for LOQ: measured concentrations are > LOQ or CO > 10LOQ, < LOQ or not reported.	15
2	Enough independent experiments/runs? >=3 or <3 independent experiments, >=3, <3 replicates or unknown?	15
3	Statistical quality is high (r2>0.85 and significant slope, number of timepoints > 6), low (not significant) or not reported (but assumed OK)	10
4	What was the chemical purity?	10
Enzymatic Activity Maintained		
5	Was the biological material characterized, and with high confidence, e.g., activity of EROD, UGT, etc?	15
6	Was the assay duration appropriate?	15
7	What was the concentration of vehicle (spiking solvent) used? Was it DMSO > 0.5% (CRITICAL FAIL)	15, FAIL if DMSO > 0.5%
8	Was a positive control or a reference chemical used?	10
Non-Metabolic Losses		
9	Were non-metabolic losses predicted to be significant based on IV-MBM? No (loss <= 20%, possible (loss > 20% and <=67% or not characterized/run/reported?	15
10	Was a negative control used and what were the (loss) results?	30
Sufficient Complementary Data		
11	Was the initial test concentration (C0) reported?	15
12	Was the cell concentration (Ccell) reported?	15
Accuracy		
13	Was initial concentration (C0) < Michaelis-Menten constant (kMM)? OR were first order kinetics confirmed?	15
14	Was the estimated assay medium concentration (Cfree) based on IV-MBM in comparison to the chemical water solubility limit (WatSol)?	10
15	What was the rate determination method? Substrate depletion (SD), confirmed or assumed or product formation (PF), confirmed or assumed.	10
16	Were the assay conditions consistent with in vivo (pH, temp, co-factors added)?	10. FAIL if Inconsistent or assumptions made
17	Was CLInVitro,Int reported or readily calculated with reported data (i.e. Ccell/Cprotein)?	5
18	Were the units presented clearly with unit conversions needed or were assumptions about the units made?	5, FAIL if unclear
19	What was the statistical difference from the control (StM)? Was BExpt significantly different from BCtrl, with high confidence (>65/100 simulations), with low confidence (<66/100 simulations) or not significantly different, low confidence (<600/100 simulations) or BExpt not significantly different from BCtrl with high confidence (>65/100 simulations)	30
20	Critical Fail for other reason (override; quality score = 0)	FAIL

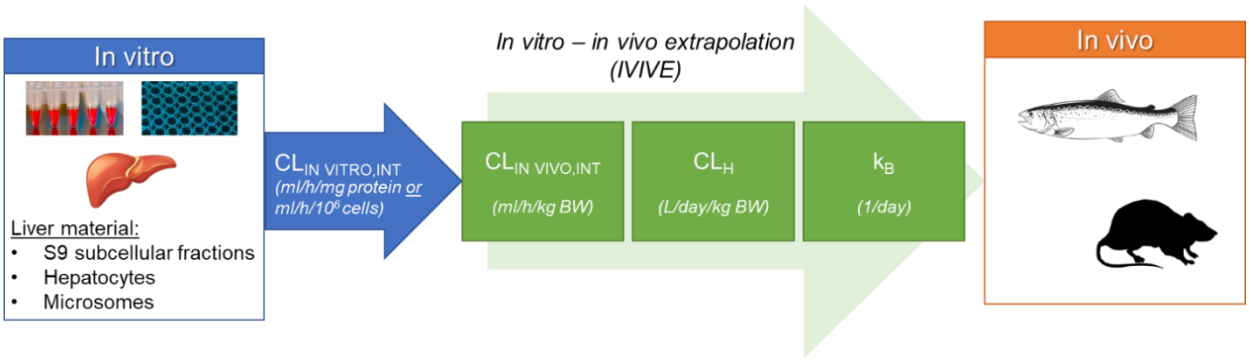
The data quality criteria for hepatocyte and microsome biotransformation studies are broadly similar and are not presented in this document. Quality criteria considered critical are indicated by Score, FAIL. If any one of these quality criteria/considerations are not met, the study is considered unreliable and given a quality score of zero. The user can still proceed with IVIVE and is given the option to include the derived biotransformation rate constant in the overall summary but the fact that the estimate is considered unreliable will be clearly documented by the BAT. If the user or a reviewer believes there is another critical failing with the study, they have the option to "override" the quality scoring by selecting "Critical Fail" in which the data reliability score is set to zero. A brief justification is expected to be provided by the user for this decision.

The DET for in vitro S9 (and the other in vitro systems) is implemented as a user form with the questions from Table 5 and appropriate options to choose from in response. The answers given by the user are recorded and displayed in the DET area of the **In Vitro S9** worksheet (below the data entry section).

In Vitro-In Vivo Extrapolation (IVIVE)

The purpose of IVIVE in the BAT is to generate whole-body biotransformation rate constants (k_B, 1/d) from the in vitro clearance data entered by the user for S9, hepatocytes or microsomal biotransformation studies. The general procedure for IVIVE is presented in Figure 18.

Figure 18. Conceptual overview of the in vitro – in vivo extrapolation (IVIVE) calculation



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 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 input sheet for IVIVE (liver S9) is presented in Figure 19. It appears when the user clicks the "*Then calculate IVIVE*" button. The user is required to address the following aspects of the IVIVE calculation:

1. Enter required parameters regarding organism physiology (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). An option to populate these parameters with a Default Parameter set is provided (See Appendices, A4. In Vitro-In Vivo Extrapolation (IVIVE)). The defaults may be edited after this option is selected.
2. Select an approach for calculating blood-water partitioning (P_{BIW}): i) equilibrium partitioning approach [64], ii) regression-based approach [67] or iii) user-entered value. The regression approach [67] was

derived for neutral organic chemicals and is not recommended for IOCs. Users are advised to select the equilibrium model or user-entered values for IOCs.

3. Enter a value the ratio of unbound fraction in blood ($f_{U,Bl}$): i) explicit calculation [61, 64], ii) $f_{U,BL}$ = 1 or iii) user-entered value.
4. Enter a value for the ratio of unbound fraction in blood and the test system (f_U): i) explicit calculation (e.g., $f_U = f_{U,Bl} / f_{U,S9}$) [61, 64], ii) f_U = 1 [60, 65] or iii) user-entered value.

Figure 19. Input sheet for IVIVE calculations (liver S9)

Additional Parameters to translate from $CL_{InVitro,INT}$ to WHOLE BODY biotransformation rate constant

Parameter	Common Symbol	Units	Value	
Liver fraction of BW	LW	g liver / g BW		
S9 content of liver	PL	mg protein / g liver		
Total Cardiac Output	QC	L/h/kg, mL/h/g		
Fraction of QC to liver	LF	-		
Lipid content of organism	$f_{L,B}$	g/g or L/L		
Protein content of organism	$f_{P,B}$	g/g or L/L		
Water content of organism	$f_{W,B}$	g/g or L/L		
Lipid content of blood	$f_{L,BL}$	g/g or L/L		
Protein content of blood	$f_{P,BL}$	g/g or L/L		
Albumin content of blood	$f_{A,BL}$	g/g or L/L		
Water content of blood	$f_{W,BL}$	g/g or L/L		
Blood-water partition coefficient	P_{BIW}	L/L		
Whole body-water partition coefficient	BCF_p	L/kg, ml/g		
Volume of Distribution	$V_{D,BL}$	L/kg, ml/g		
Fraction unbound in whole blood	$f_{W,BL}/P_{BIW}$			←3.
Fraction unbound	$f_{U,BL}/f_{U,S9}$			←4.
Invivo intrinsic Clearance	$CL_{InVivo,Int}$	mL/h/g body weight		
Hepatic Clearance, water ratio correction factor	CL_H	L/h/kg, mL/h/g		
Whole body biotransformation rate constant	k_B	h^{-1}		

Select Options to Calculate: Return to In Vitro DET to continue

1. For IVIVE parameters, enter or select:

○ User ○ Default Organism

Organism and blood compositions used to calculate BCF_p and if Equilibrium Partitioning option selected, P_{BLW}

2. To calculate P_{BIW}:

○ Equilibrium Partitioning ○ Fitzsimmons et al. (2001)

Return to In Vitro DET

The equations and options implemented in the BAT for IVIVE are documented in the Appendices (A4. In Vitro-In Vivo Extrapolation (IVIVE)). Additional data requirements for the IVIVE calculations are also discussed further. These include data such as body and blood composition, total cardiac output, and fraction of blood flow to liver and are based on published values, e.g., [61, 64, 68]. **It is recognized that other IVIVE models have been developed or are under development, e.g., [69, 70], and future versions of the BAT may allow for incorporation of other approaches and parameterizations**.

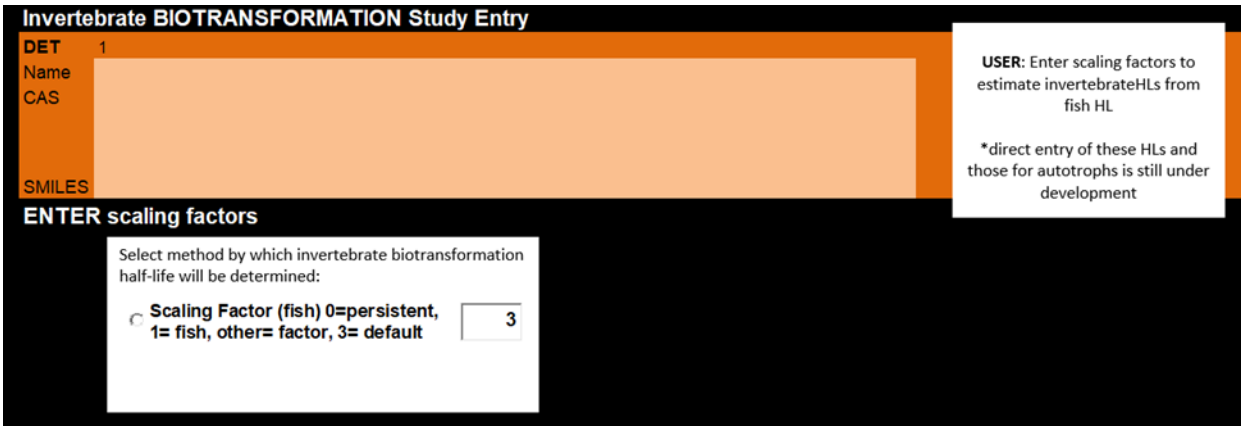
Interspecies and Interclass Extrapolation of Biotransformation Data

The BAT allows the user to enter and process in vitro and in silico biotransformation rate constant data for both fish and mammals. As discussed above, biotransformation rate constant data are normalized to a given body size and temperature (e.g., 0.01 kg and 15 °C for fish). For modeling purposes, the normalized biotransformation rate constant for fish ($k_{B,N}$) is scaled up or down by size for all fish species that are simulated by the built-in BAT models. Likewise, the $k_{B,N}$ for mammals is scaled up or down by size for all mammalian species. There is uncertainty regarding the normalization process; however, performing the normalization (rather than not performing the normalization) is consistent with common practice in the pharmaceutical and veterinary sciences.

Although the general expectation is that mammals exhibit greater metabolic competence than fish (i.e., larger biotransformation rate constants for a given size), there is no established approach to extrapolate biotransformation rate data between these classes of organisms. Accordingly, unless class-specific data are entered by the user, the chemical is assumed to be persistent in that class of organism. In other words, regardless of the biotransformation rate constant established for fish, the biotransformation rate constant for mammals will be zero unless mammalian biotransformation rate data are entered (and vice versa). In such cases, it is possible that a chemical will be predicted to be "not bioaccumulative" in fish/aquatic food webs but "bioaccumulative" in mammals/terrestrial food webs (and vice versa). Modeling results generated from the BAT in such cases should therefore be interpreted cautiously and with full recognition of the role of biotransformation. To address data gaps in this often-critical parameter, the user may wish to assume rates from one class (e.g., "fish") are applicable to the other (e.g., "mammals"); however, these assumptions need to be clearly stated for transparency.

Biotransformation rate data in lower-order taxa, including aquatic/benthic invertebrates and autotrophs such as vegetation and phytoplankton (algae), are sparse; however, chemical biotransformation in these organisms, which also constitutes food sources for higher-order taxa (i.e., fish, birds, mammals), may be significant. BAT Ver.2.02 has included a relatively simple way to address current data gaps for estimating biotransformation rate constants for invertebrates by allowing the user to assume biotransformation (or not) for invertebrates. After the user has finished adding biotransformation information for fish and/or mammals, they must click the "4. Define Invert Biotrans HLs" button before continuing. Figure 20 shows the interface of the **Invert-BioTrans** sheet. By default, the scaling factor is set to 3 for invertebrates. The average fish biotransformation half-life normalized to a 10 g organism at 15 °C is multiplied by these scaling factors and then scaled to body mass to estimate the biotransformation half-life in those organisms. This scaling factor method implies that "all else being equal", the biotransformation half-life for a chemical in an invertebrate is three times longer (slower) than in a fish of the same mass and system temperature. The user may always set the scaling factor to "0" to create the assumption that the chemical is "totally persistent" in an invertebrate. Direct entry of biotransformation half-lives for aquatic/benthic invertebrates and autotrophs may be implemented in a future version of the BAT.

Figure 20. Invertebrate biotransformation entry sheet



Addressing Uncertainty in Available Biotransformation and Dietary Absorption Efficiency Data

All estimated whole-body biotransformation rate constants for fish, mammals, invertebrates and dietary absorption efficiency values for fish and mammals are summarized on the **Chemical Property** worksheet along

with the associated reliability scores. For subsequent calculations performed by the BAT (e.g., in silico bioaccumulation assessment), the reliability and method weighting score-weighted mean of the natural logarithm-transformed value ($\ln k_{B,N,AVG}$) is calculated and used, as documented in the Appendices (A5. Calculation of Average Biotransformation Rates). Following Slob [71], the BAT also calculates a confidence factor (CF) based on the available data, which represents the upper and lower bound values (95% confidence interval). It uses the maximum reported biotransformation value to estimate the 97.5th percentile, the minimum reported biotransformation value to estimate the 2.5th percentile and the reliability score-weighted mean to determine the average spread, expressed as CF, of the reported data using this calculation:

$$\sigma = \left(\frac{\sum_{i=1}^{n}(\ln k_{B,Ni} - (\ln k_{B,N,AVG})}{n-1}\right)$$

$$CF = \exp\left(1.96 * \sigma^2\right)$$

For example, a confidence factor (CF) of 3 means that BAT calculations are also conducted using biotransformation rates equal to $k_{B,N,AVG} \cdot 3$ and $k_{B,N,AVG} / 3$ to determine the maximum and minimum estimated BCFs and BMFs generated by the BAT. This process is applied to fish, to mammals and for any entered dietary absorption efficiency (E_D) for fish, herbivores, and carnivores. If a single biotransformation rate or E_D value is assessed, then the CF for that parameter equals 1. This value does not indicate that the value used is necessarily the "most confident" parameter, but rather that there are not enough data to estimate the distribution.

The reliability score-weighted mean biotransformation rate and CF are also summarized on the **Chemical Property** worksheet. Biotransformation rate data with a reliability score of "0" (e.g., "critical fails") are displayed in the k_B summary in the **Chemical Property** worksheet with an adjacent "*****". However, such data **are not used** in the averages or confidence factors.

Bioaccumulation Data

In the third stage of the BAT workflow, bioaccumulation data from multiple sources (in vivo, in silico; laboratory, field) can be entered by the user and included in the QWOE. The development of standardized testing guidance has been relatively more involved for laboratory data endpoints (i.e., BCF, BMF) compared to some of the other LoE (i.e., field data and QSARs). Hence the data quality evaluation process can be in some cases a little more time-consuming. As with the other LoE, there are two tasks to complete bioaccumulation data entry, i) key test details and quantitative information and ii) the data quality (**reliability**) assessment.

Laboratory BCF Data (Fish)

The OECD 305 test guidelines for aquatic bioaccumulation testing [4] are well-established and recognized by regulatory authorities and therefore the DETs for laboratory BCF data were largely based on the reporting requirements outlined in the Technical Guidance Document. However, the objective of the DET for laboratory BCF data is **not to determine whether a test satisfies all OECD 305 requirements** but instead seeks to provide a more general assessment of data reliability. The DET for laboratory BCF data reflects current REACH guidance documents and was also based on previous BCF and BAF database development conducted and published by Arnot et al. [3]. Once the user has completed the data entry and reliability assessment activities, various calculations and checks are automatically conducted by the BAT when the "*Assess Input and Reliability"* button is pressed.

Key Test Details and Quantitative Information

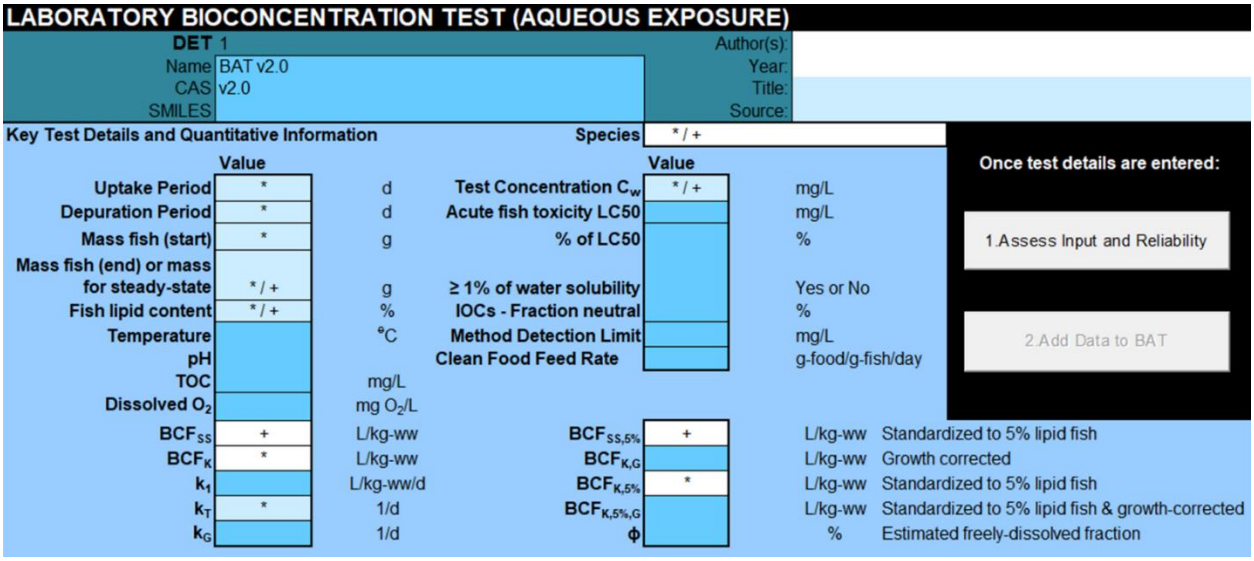
The purpose of this section in the BAT is to collect all information relevant for calculating the bioconcentration factors including test conditions and kinetic information (if available). A screen capture of this section is included here as **Figure 21**. There are two types of empirical BCF data the user can enter, i) a steady-state BCF (BCF_{SS}, i.e., $C_{organism}/C_{water}$) and ii) a kinetic BCF (BCF_K).

The mandatory user inputs include the author and year (citation) and the species studied. Mandatory inputs to calculate a steady-state BCF (BCF_{SS}) are i) fish mass (end of exposure), ii) total lipid content, iii) test concentration and/or, iv) the BCF_{SS} value itself. These mandatory inputs are indicated by the cells with a "**+**" symbol.

The mandatory user inputs for a kinetic BCF (BCF_K) are i) uptake period and depuration period, ii) mass of fish at beginning and end of exposure, iii) total lipid content, iv) total elimination rate constant and v) the test concentration or vi) the BCF_K. These inputs are indicated by the cells with a "*****" symbol. The user can also enter other toxicokinetic data which, if absent, are estimated by the BAT (see below).

The user is also able to enter information regarding test conditions such as temperature, pH, total organic carbon in the water column (TOC) and dissolved O_2 and toxicological data (LC50) to which the reported test concentration is compared to indicate whether the organisms were more likely to have experienced adverse effects during exposure. The relationship between the total water concentration (C_{WT}), as is commonly measured, and the freely-dissolved water concentration, which is bioavailable for uptake, is expressed by the parameter $\phi = C_{WD}/C_{WT} = 1/(1 + K_{POC}\chi_{POC} + K_{DOC}\chi_{DOC})$, where χ_{POC} and χ_{DOC} are the concentrations (kg/L) of particulate and dissolved organic carbon in the water respectively. If the user enters data on TOC, the estimated freely-dissolved fraction (ϕ) will also be calculated when the "*Assess Input and Reliability*" button is clicked at the DET completed (see below). The BAT also compares the reported test concentration to water solubility and, if the chemical is an IOC, calculates the fraction in neutral form at the bulk water pH.

Figure 21. Key test details and quantitative information—Laboratory BCF (Fish)



Steady-State BCF (BCF_{SS})

The steady-state BCF is calculated as the ratio of the concentration in the organism (C_B) and the concentration of the chemical in 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 be stable (i.e., achieve the steady-state value). These considerations are included in the DETs but otherwise the BAT conducts no additional checks. To include a BCF_{SS} in the BAT assessment, the user simply enters the value reported in the test.

Kinetically-Derived BCF (BCF_K)

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. To include a BCF_K in the BAT assessment, the user is asked to enter the k_1 and k_T values derived from the study being considered. The user can enter the growth dilution rate constant (k_G, 1/d). As required, the user is asked to select between the manually entered data and the BAT generated data.

Additional BCF Metrics Calculated by the BAT

If the required data are available, the BAT automatically completes several calculations when the "*Assess Input and Reliability*" button is pressed. The purpose of these calculations is to derive additional BCF metrics expected to be of importance to regulators such as i) the lipid-standardized steady-state BCF ($BCF_{SS,L}$), ii) the growth-corrected kinetic BCF ($BCF_{K,G}$), iii) the lipid-standardized kinetic BCF ($BCF_{K,L}$) and iv) the growth-corrected and lipid-standardized kinetic BCF ($BCF_{K,L,G}$). The user can enter these data manually and the BAT will then compare the user-entered values to those calculated from other information provided. Note that **lipid-standardization** in the BAT is the conversion of a wet-weight BCF for a given lipid content (e.g., 3.5%) to the wet-weight BCF for a 5% lipid content fish. In other words, metrics reported as **lipid standardized** are wet weight values for a 5% lipid content organism. All equations for the Lab BCF calculations are documented in the Appendices (A6. Bioaccumulation Data (Empirical)).

Data Reliability Assessment (Data Evaluation Template)

The data quality criteria and considerations for an empirical laboratory BCF study are based on OECD guidance documents [4] and are summarized in **Table 6**.

Data quality criteria considered critical are indicated by Pass/Fail. If any of these quality/criteria/considerations are not met, the study is considered unreliable and given a quality score of zero. If the user believes there is another critical failing with the study, they have the option to "override" the quality scoring by selecting "Critical Fail" in which the data reliability score is set to zero. A brief justification is expected to be provided.

Table 6. Data quality considerations for empirical laboratory BCF studies (Fish)

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

The user is also able to enter information regarding test conditions such as temperature, pH, total organic carbon in the water column (TOC) and dissolved O_2 and toxicological data (LC50) to which the reported test concentration is compared to indicate whether the organisms were more likely to have experienced adverse effects during exposure. The relationship between the total water concentration (C_{WT}), as is commonly measured, and the freely-dissolved water concentration, which is bioavailable for uptake, is expressed by the parameter $\phi = C_{WD}/C_{WT} = 1/(1 + K_{POC}\chi_{POC} + K_{DOC}\chi_{DOC})$, where χ_{POC} and χ_{DOC} are the concentrations (kg/L) of particulate and dissolved organic carbon in the water respectively. If the user enters data on TOC, the estimated freely-dissolved fraction (ϕ) will also be calculated when the "*Assess Input and Reliability"* button is clicked at the DET completed (see below). The BAT also compares the reported test concentration to water solubility and, if the chemical is an IOC, calculates the fraction in neutral form at the bulk water pH. Entry of information about sediment concentrations and % porewater respired is available and important to document, however, calculations in this version of the BAT only considers exposure to the water column phase. Future work is planned to incorporate exposure of aquatic invertebrates to sediment concentrations in simulated laboratory experiments.

Laboratory BCF Data (Invertebrates)

There is increasing regulatory interest in bioaccumulation data for invertebrates and in response the BAT Ver.2.0 was updated to allow laboratory BCF data for invertebrates to be entered by the user. Although there are no OECD test guidelines for testing invertebrates for BCF equivalent to the guidelines for fish (OECD 305), the same general considerations are relevant for assessing data reliability. The DET for laboratory BCF data for invertebrates was therefore based on the DET for fish. Once the user has completed the data entry and reliability assessment activities, various calculations and checks are automatically conducted by the BAT when the "*Assess Input and Reliability"* button is pressed.

Key Test Details and Quantitative Information

The purpose of this section in the BAT is to collect all information relevant for calculating the bioconcentration factors including test conditions and kinetic information (if available). A screen capture of this section is included here as Figure 22. As for fish, there are two types of empirical BCF data the user can enter for invertebrates, i) a steady-state BCF (BCF_{SS}, i.e., $C_{organism}/C_{water}$) and ii) a kinetic BCF (BCF_K).

The user may directly enter any known BCF_{SS}, $BCF_{SS,5\%}$, BCF_K, $BCF_{K,G}$, $BCF_{K,5\%}$, $BCF_{K,5\%,G}$. It is encouraged that the user also enter any pertinent study information that may be used to recalculate the entered values as a "check". There are unlocked workspaces beginning at (or around) cell O33 for the user to include any conversion/calculations necessary to standardize the data entered into the BAT on this sheet. Additionally, an unlocked worksheet named "Work Area" can be utilized.

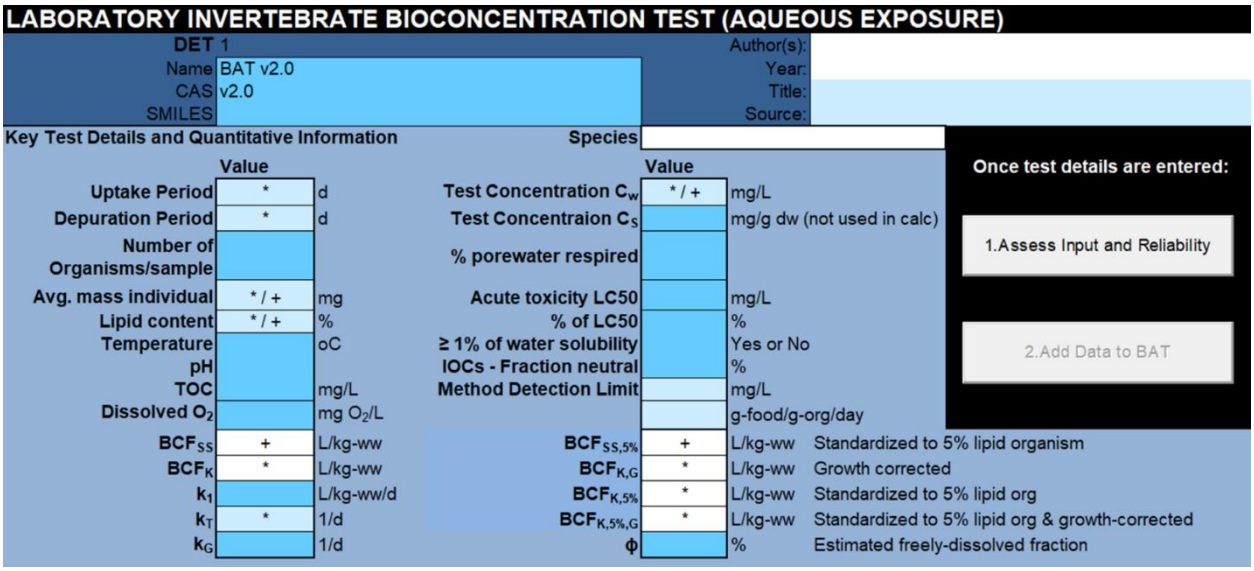
The mandatory user inputs to calculate a steady-state BCF (BCF_{SS}) are i) average mass per individual (mg), ii) total lipid content, iii) test concentration and of course, iv) the BCF_{SS} value itself. These mandatory inputs are indicated by the cells with a "**+**" symbol.

The mandatory user inputs to calculate a kinetic BCF (BCF_K), other than author, year, and species, are i) uptake period and depuration period, ii), average mass per individual (mg) iii) total lipid content, iv) total elimination rate constant and v) the test concentration. These inputs are indicated by the cells with a "*****" symbol. The user can also enter other toxicokinetic data which, if absent, are estimated by the BAT (Figure 22).

Steady-State BCF (BCF_{SS})

The steady-state BCF is calculated as the ratio of the concentration in the organism (C_B) and the concentration of the chemical in 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 invertebrates must be stable (i.e., achieve the steady-state value). These considerations are included in the DETs but otherwise the BAT conducts no additional checks. To include a BCF_{SS} in the BAT assessment, the user simply enters the value reported in the test.

Figure 22. Key test details and quantitative information—Laboratory BCF (Invertebrates)



Kinetic BCF (BCF_K)

The kinetically-derived BCF is calculated as the ratio of the 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. To include a BCF_K in the BAT assessment, the user is asked to enter the k_1 and k_T values derived from the study being considered. The user can enter the growth dilution rate constant (k_G, 1/d) if it is known. As required, the user is asked to select between the manually entered data and the BAT generated data if the entered and generated values are different.

Additional BCF Metrics Calculated by the BAT

If the required data are available, the BAT automatically completes several calculations when the "*Assess Input and Reliability*" button is pressed. The purpose of these calculations is to derive additional BCF metrics expected to be of importance to regulators such as i) the lipid-standardized steady-state BCF ($BCF_{SS,5\%}$), ii) the growth-corrected kinetic BCF ($BCF_{K,G}$), iii) the lipid-standardized kinetic BCF ($BCF_{K,5\%}$) and iv) the growth-corrected and lipid-standardized kinetic BCF ($BCF_{K,5\%,G}$). The user can enter these data manually and the BAT will then compare the user-entered values to those calculated from other information provided. Note that **lipid-standardization** in the BAT is the conversion of a wet-weight BCF for a given lipid content (e.g., 3.5%) to the wet-weight BCF for a 5% lipid content invertebrate. In other words, metrics reported as

lipid standardized are wet weight values for a 5% lipid content organism. All equations for the Lab BCF calculations are documented in the Appendices (A6. Bioaccumulation Data (Empirical)).

Data Reliability Assessment (Data Evaluation Template)

The data quality criteria and considerations for an empirical laboratory BCF study using invertebrates is based on the DET for fish (see Table 6).

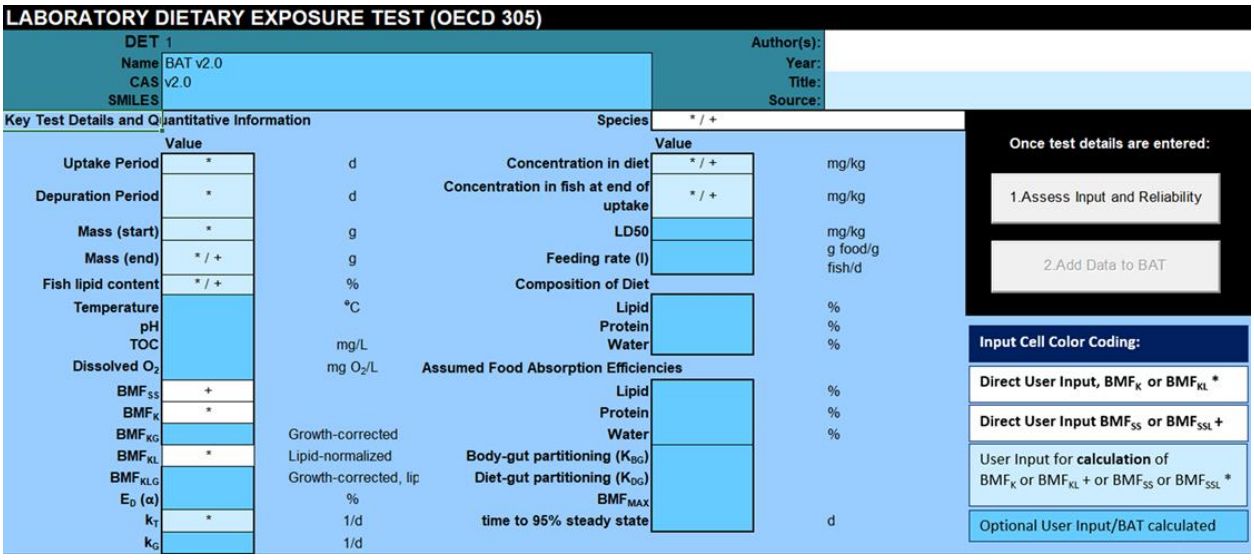
Laboratory BMF Data (Fish)

The OECD 305 test guidelines for the dietary exposure of aquatic organisms [4] are well-established and recognized by regulatory authorities and therefore the DETs for laboratory BMF data were largely based on the reporting requirements outlined in the Technical Guidance Document. However, as with the laboratory BCF DET, the objective of the DET for laboratory BMF data is **not to determine whether a test satisfies all OECD 305 requirements** but instead seeks to provide a more general assessment of quality/reliability. The DET for laboratory BMF data reflects current REACH guidance documents and was also based on previous BMF database development conducted and published by Quinn and Arnot [72]. Once the user has completed the data entry and reliability assessment activities, various calculations and checks are automatically conducted by the BAT when the "*Assess Input and Reliability"* button is clicked.

Key Test Details and Quantitative Information

The purpose of this section in the BAT is to collect information relevant for calculating BMFs including test conditions and kinetic information. A screen capture of this section is included here as Figure 23. Note that this input sheet is designed to handle steady-state and kinetic BMF data, e.g., OECD 305) [4], as reflected in the user inputs.

Figure 23. Key details and quantitative information—Laboratory BMF (Fish)



The user may directly enter any known BMF_{SS}, BMF_K, $BMF_{K,L}$. It is encouraged that the user also enter any pertinent study information that may be used to recalculate the entered values as a "check" or simply be a record of the study conditions. There are unlocked workspaces beginning at cell O35 for the user to include any conversion/calculations necessary to standardize the data entered into the BAT on this sheet. Additionally, an unlocked worksheet named "Work Area" can be utilized.

The mandatory user inputs for the Lab BMF input sheet are author, year, and species. To calculate a steady-state BMF the user must enter i) mass of fish at end of exposure, ii) total lipid content, iii) the concentration of chemical in the diet, iv) the concentration of chemical in the fish at the end of the uptake phase and/or the steady-state BMF itself.

The mandatory user inputs for the Lab BMF input sheet for a kinetic BMF include i) uptake and depuration period, ii) mass of fish at the start and end of exposure, iii) total lipid content, iv) the total elimination rate constant (k_T), v) the concentration of chemical in the diet and vi) the concentration of chemical in the fish at the end of the uptake phase. It is strongly recommended that the user enter the reported feeding rate. The BAT will assume a default value (I = 0.02 g food / g fish /d) in the absence of a test-specific value.

As with the laboratory BCF DET, the user is also able to enter information regarding test conditions including temperature, pH, TOC and dissolved O_2. This information is used by the BAT to calculate various BMF metrics once the "*Assess Input and Reliability"* button is clicked. Although not required for the key BMF calculations, data on dietary composition and absorption efficiencies can also be entered by the user. These data are used by the BAT to calculate the additional BMF-related metrics described below.

Key BMF Metrics Calculated by the BAT

A series of BMF calculations are conducted by the BAT upon pressing the "*Assess Input and Reliability"* button assuming the required data have been entered by the user. Note that the user can enter these data manually and the BAT will then ask the user the keep those data or use the calculated values. The purpose of these calculations is to derive various metrics expected to be of importance for bioaccumulation assessment such as i) the wet weight biomagnification factor (BMF), ii) the growth-corrected kinetic BMF (BMF_G), iii) the lipid-normalized BMF (BMF_L) and iv) the growth-corrected and lipid-normalized BMF ($BMF_{L,G}$). The chemical absorption efficiency (E_D or α), which is required to calculate kinetic BMFs from the test data is also calculated by the BAT. The equations implemented in the BAT are documented in the Appendices (A6. Bioaccumulation Data (Empirical)).

Additional BMF-Related Metrics Calculated by the BAT

To provide additional insight into the dietary uptake and bioaccumulation of the chemical of interest, the BAT also provides the following metrics: i) body-gut partition coefficient (K_{BG}), ii) diet-gut partition coefficient (K_{DG}), and iii) theoretical maximum BMF, excluding biotransformation (BMF_{MAX}) [73].

Body-gut and diet-gut partition coefficients give information about the relative sorptive capacities of the body and diet compared to digested food. Larger values of these partition coefficients imply greater biomagnification *potential*. BMF_{MAX} is the expected wet weight BMF based on the gastrointestinal magnification caused by the reduction in volume (G_D vs G_F) and sorption capacity (Z_D vs Z_G) that occurs during the digestion process [73]. Growth dilution is included in this calculation, but biotransformation is not. Accordingly, laboratory BMFs substantially lower than the BMF_{MAX} suggest that biotransformation could be an important factor mitigating the bioaccumulation potential of the chemical whereas laboratory BMFs ~ equal to the BMF_{MAX} indicate that the chemical is well absorbed and persistent. Laboratory BMFs substantially lower than BMF_{MAX} could also mean that the passive uptake (absorption) of the chemical is hindered (e.g., steric factors) in comparison to other chemicals of similar hydrophobicity. Active transport back into the gut lumen could also be a factor.

Data Reliability Assessment (Data Evaluation Template)

The data quality criteria for an empirical laboratory BMF study are based on OECD guidance documents [4] and are summarized in **Table 7**. Data quality criteria considered critical are indicated by Pass/Fail. If any

one of these quality/criteria/considerations are not met, the study is considered unreliable and given a reliability score of zero. If the user believes there is another critical failing with the study, they have the option to “override” the quality scoring by selecting “Critical Fail” in which the data reliability score is set to zero. A brief justification is expected to be provided.

The DET for Laboratory BMF study is implemented as a user form with the questions from Table 7 and appropriate options to choose from in response. The answers given by the user are recorded and displayed in the DET area of the Laboratory BMF study (below data entry section). This display is only revealed after the user has completed the user form. The user form can be re-opened, and the answers revised.

NOTE: If enough data are provided by the user in the key test details and quantitative information section, the BAT will automatically complete some of the data quality considerations.

Table 7. Data quality considerations for empirical laboratory BMF studies (Fish)

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

Laboratory Toxicokinetic (TK) data (Mammals)

Although dietary uptake testing in mammals like laboratory BMF tests with fish (OECD 305) are rare, there are studies reporting toxicokinetic (TK) data that are useful for B assessment. Specifically, the total elimination rate constant (k_T) or half-life (HL_T) derived from mammalian TK studies can be used to estimate the biomagnification factor (BMF) using the following (kinetic) expression,

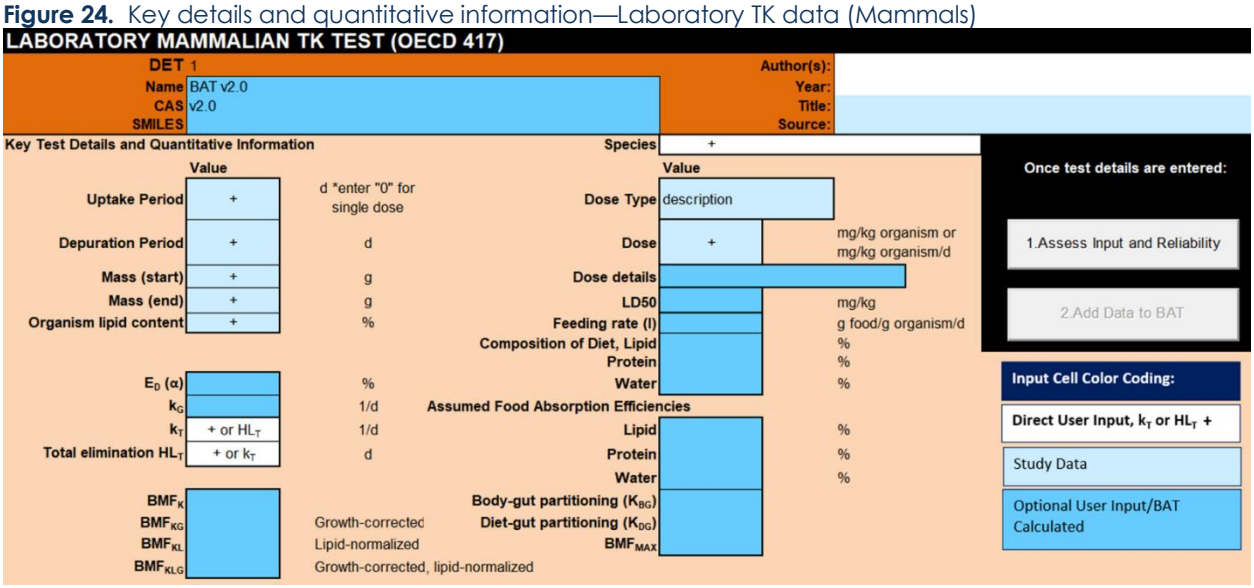
$$BMF_K = \frac{I \cdot E_D}{k_T} = \frac{k_D}{k_T}$$

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 gut. Note that k_T can be estimated from half-life as $\ln 2/HL_T$). The BMF_K can then be lipid-normalized and growth-corrected as necessary following standard approaches.

Key Test Details and Quantitative Information

The purpose of this section in the BAT is to collect all information relevant for calculating a kinetic BMF from TK data derived from laboratory testing (e.g., OECD 417 [74]). A screen capture of this section is included here as Figure 24. Both single and repeated dose exposures can be entered by the user but in both cases only the total elimination kinetics are used to calculate the kinetic BMF.

Figure 24. Key details and quantitative information—Laboratory TK data (Mammals)



The user may directly enter any known k_T or HL_T. It is encouraged that the user also enter pertinent study information that may be used to recalculate the entered values as a "check" or simply be a record of the study conditions. There are unlocked workspaces beginning at cell O35 for the user to include any conversion/calculations necessary to standardize the data entered into the BAT on this sheet. Conversions from clearance rates (CL), volume of distribution (V_D) and area under the curve (AUC) can automatically be made in this area, with the equations shown. An unlocked worksheet named "Work Area" can be utilized.

The mandatory user inputs to calculate a BMF_K are i) uptake period and depuration period, ii), average mass of the laboratory animal at the start and end of exposure iii) total lipid content (if empty then a generic value of 8.98% is used), iv) total elimination rate constant or half-life and v) the test concentration (dose). These inputs are indicated by the cells with a "**+**" symbol. The user has the option to enter the feeding rate

(I) or leave blank so that a standardized default assumption (0.05g food/g organism/day) is used. The user can also enter other toxicokinetic data which, if absent, are estimated by the BAT.

Key BMF Metrics Calculated by the BAT

Various BMF calculations are automatically conducted by the BAT upon pressing the "*Assess Input and Reliability"* button assuming the required data have been entered by the user. In addition to the wet weight kinetic BMF (BMF_K), the BAT also provides i) the growth-corrected wet weight kinetic biomagnification factor (BMF_{KG}), ii) the lipid-normalized kinetic BMF (BMF_{KL}) and iii) the lipid-normalized and growth-corrected kinetic BMF (BMF_{KLG}). The chemical absorption efficiency (E_D or α), which is required to calculate kinetic BMFs from the test data is also calculated by the BAT if not already entered by the user as an optional input.

Additional BMF-Related Metrics Calculated by the BAT

To provide additional insight into the dietary uptake and bioaccumulation of the chemical of interest, the BAT also provides the following metrics: i) body-gut partition coefficient (K_{BG}), ii) diet-gut partition coefficient (K_{DG}), and iii) theoretical maximum BMF, excluding biotransformation (BMF_{MAX}) [73].

Body-gut and diet-gut partition coefficients give information about the relative sorptive capacities of the body and diet compared to digested food. Larger values of these partition coefficients imply greater biomagnification *potential*. BMF_{MAX} is the expected wet weight BMF based on the gastrointestinal magnification caused by the reduction in volume (G_D vs G_F) and sorption capacity (Z_D vs Z_G) that occurs during the digestion process [73]. Growth dilution is included in this calculation, but biotransformation is not. Accordingly, laboratory BMFs substantially lower than the BMF_{MAX} suggest that biotransformation could be an important factor mitigating the bioaccumulation potential of the chemical whereas laboratory BMFs ~ equal to the BMF_{MAX} indicate that the chemical is well absorbed and persistent. Laboratory BMFs substantially lower than BMF_{MAX} could also mean that the passive uptake (absorption) of the chemical is hindered (e.g., steric factors) in comparison to other chemicals of similar hydrophobicity. Active transport back into the gut lumen could also be a factor.

Data Reliability Assessment (Data Evaluation Template)

The data quality criteria for an empirical laboratory BMF study are based on OECD 305 Technical Guidance [4] and published BMF data quality assessment methods [72] are summarized in **Table 8**. Data quality criteria considered critical are indicated by Pass/Fail. If any one of these quality/criteria/considerations are not met, the study is considered unreliable and given a reliability score of zero. If the user believes there is another critical failing with the study, they have the option to "override" the quality scoring by selecting "Critical Fail" in which the data reliability score is set to zero. A brief justification is expected to be provided.

Table 8. Data quality considerations for laboratory TK data (mammals)

#	Quality Criterion/Consideration	Maximum Score
1	Was the parent/metabolite reported?	5
2	Sample size reported?	10
3	Number of sampling time points reported?	10
4	Chemical purity of the administered compound reported?	5
5	Clarity of reported rate units	10
6	Relevant biological information reported? e.g., strain/sex/age	5
7	Body weight/mass reported?	5
8	Dose reported with units?	10
9	Route of administration reported?	5
10	Test duration reported?	5
11	Dosing reported?	5
12	Frequency of dosing reported?	5
13	Testing of which tissue reported?	5
14	Vehicle used reported?	5
15	Was there a control group?	5
16	Was there an indication of toxicity?	10
17	Was there a toxicokinetic model presented?	5
18	How was the rate of elimination (kT) / half life (HLT) determined?	10
20	Critical Fail for other reason (override; quality score = 0)	Fail

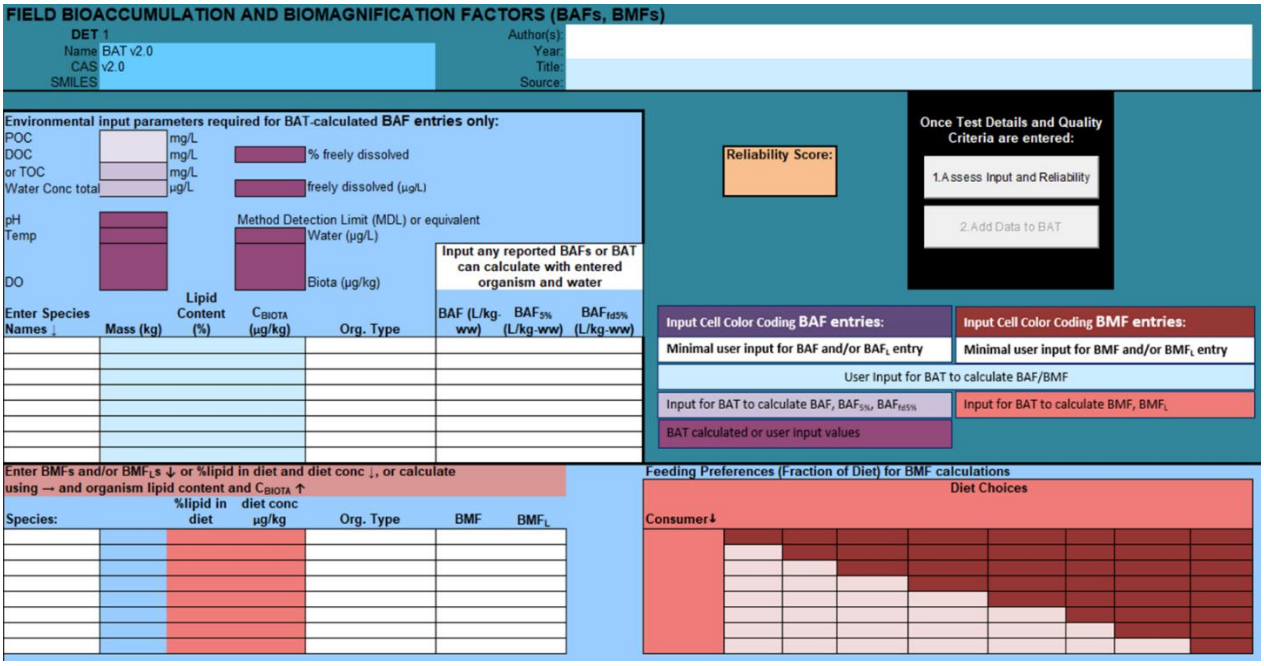
Field BAF and BMF Data

The Field BAF and BMF data input sheet provides an opportunity for the user to enter data from field sampling campaigns as part of the overall bioaccumulation assessment. In this case, the field data may not be amenable to calculating a TMF (e.g., because trophic position cannot accurately be determined) but still allows BAFs and BMFs to be assessed/calculated.

Key Details and Quantitative Information

The user can enter data characterizing: i) environmental/exposure conditions, ii) the organisms sampled, and iii) feeding preferences (food web structure), and/or iv) bioaccumulation metrics as outlined in the following sections. A maximum of eight organisms can be entered per sheet. If more than eight organisms are reported then a second (or third) entry sheet can be filled out. The user must click the "*1. Assess Input and Reliability"* and "*2.Add Data to BAT"* buttons as each sheet is filled out. The key data entry form is shown in Figure 25.

Figure 25. Key details and quantitative information—Field BAF and BMF study



Environmental/Exposure Conditions

The mandatory user inputs for environmental / exposure conditions to calculate field BAF and BMF data are i) Concentration of chemical in the water column (total) (µg/L), ii) TOC (mg/L), and iii) pH and temperature of the water column. Particulate and dissolved organic carbon in the water column (POC or DOC, mg/L) can also be included if available. In addition to these inputs, organism data as described below are necessary.

Organisms

The mandatory organism inputs for field BAF and BMF data are i) mass (kg), (ii) total lipid content and iii) concentration of the chemical in the organism (wet weight, µg/kg) and iv) organism type.

Feeding Preferences (Matrix)

The final mandatory input is the feeding preferences (matrix) for the organisms included in the field study. Feeding preferences are entered as fractions of total diet as shown on the bottom right-hand side of **Figure 25**. Note that fractions of total diet for each organism included must sum to 1.

Bioaccumulation Metrics

The user may enter only the reported BAFs (total water, freely-dissolved and/or lipid-standardized) and/or BMFs (wet weight or lipid-normalized). Any data entered in the other sections will aid in checking the entered data values against calculated ones and/or calculating other forms of the bioaccumulation metrics.

Calculations Conducted by the BAT

The BAT calculates the following parameters and bioaccumulation metrics using data provided by the user:

- BAF (total water)

- BAF_L (total water, lipid-normalized)
- $BAF_{fd,L}$ (based on freely-dissolved water concentration, lipid-normalized)
- BMF (wet weight)
- BMF_L (lipid-normalized BMF)

Data Reliability Assessment (Data Evaluation Template)

The data quality criteria for field BAF and BMF data are presented in Table 9. They are based on considerations for laboratory data and field considerations, e.g., [3, 8] and are all associated with a numerical score. If the user believes there is another critical failing with the study, they have the option to override the quality scoring based on the other quality criteria/considerations. A brief justification is expected to be provided. The scoring is automatically completed based on user responses when the "*Assess Input and Reliability"* button is pressed.

Table 9. Data quality considerations for field BAF and BMF data

#	Quality Criterion/Consideration	Maximum Score
1	Field blanks used in the sampling?	30
2	Randomized sampling method employed?	10
3	Water (for BAF) and dietary (for BMF) samples used in B metric co-located and considered representative of the exposures?	30
4	Biological and environmental (e.g. water) samples used in B metric obtained in same year and season?	30
5	Confidence that steady-state is approximated (e.g., +/-~20%) on a score of 0-30 (30 being analytical confirmation of this assumption):	30
6	For neutral chemical BMFs: lipid contents in predator and prey are reported?	30
7	Analytical standards used in the analysis?	20
8	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)?	50
9	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)?	50
10	How are the measurements below the MDL addressed? Statistically, by replacement or unknown?	10
11	Are sampled species names reported?	5
12	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)?	5
13	Is environmental temperature reported?	5
14	For ionizables: was pH reported?	5
15	Critical Fail for other reason (override; quality score = 0)	Fail

The DET is accessed by clicking the "*1. Assess Input and Reliability*" button on the **Field BAF BMF** worksheet that the user can interact with to respond to questions.

Field TMF Data

The **Field TMF** sheet provides an opportunity for the user to enter measured TMF data from field sampling campaigns as part of the overall bioaccumulation assessment. In this case, trophic positions of the organisms are sufficiently characterized in the study to allow for the TMF calculation.

The general equations for calculating trophic magnification factors (TMFs) are presented below [9]:

$$TMF = 10^{b}$$

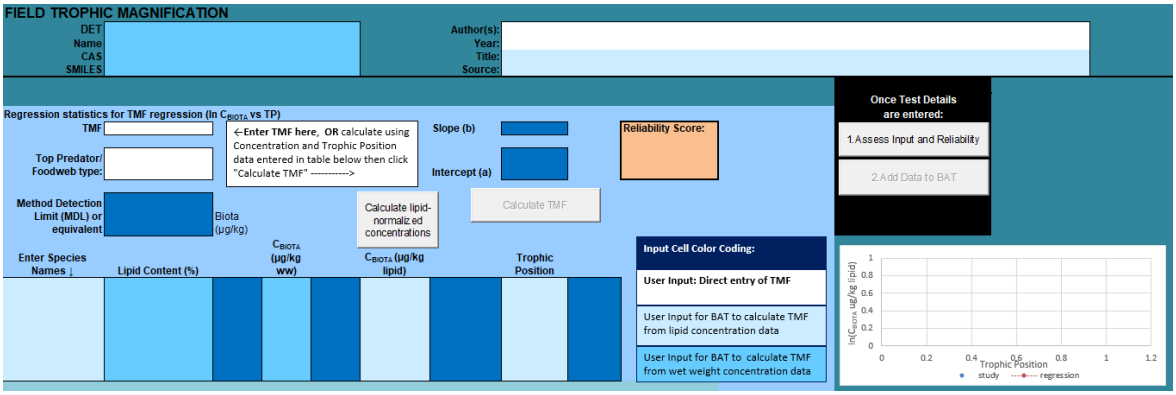
$$TMF = e^{b}$$

where *b* is the slope of the regression between the $\log_{10}$ concentration or the natural log (ln) concentration of chemical in the organism (lipid-normalized) and trophic level (TL). The TMF therefore represents the average factor by which concentrations change over the defined food web. TMFs > 1 indicate biomagnification and TMFs <1 indicate biodilution.

Key Details and Quantitative Information

The input sheet for Field TMF data is presented in Figure 26. Details including information about the method detection limit are not required but are helpful in determining the quality of study output as environmental levels may be below method detection limits, thus rendering the values used in the regressions less meaningful.

Figure 26. Input sheet for field TMF data



Option 1. The user can simply enter the TMF reported in the study of interest and identify the top predator/food web type from the pull-down menu and click on the "*Assess Input and Reliability*" button to complete the reliability scoring (see below).

Option 2. The user can enter the appropriate data and the BAT will calculate the TMF (recommended, for transparency). In this case, the mandatory user input for field TMF data are: i) total lipid content, ii) measured concentrations (wet weight), iii) trophic position (e.g., as determined by stable isotope analyses), and iv) identify the top predator/food web type from the pull-down menu. The user can also enter lipid-normalized concentrations, but the BAT will also complete these calculations if the *Calculate lipid-normalized concentrations* button. Once these data are entered or calculated, the user must click on the "*Calculate TMF*" button. The BAT will then calculate the slope (b) and intercept (a) of the data entered in the column for lipid-normalized concentrations in biota. The TMF is then presented to the user on the same data entry sheet and on the **Results** sheet. The user can then complete the reliability scoring for this LoE (see below).

Data Reliability Assessment (Data Evaluation Template)

The data quality criteria for field TMF data are presented in Table 10. They are based on considerations for laboratory data and field considerations, e.g., [3, 8], and are all associated with a numerical score. If the user or a reviewer believes there is another critical failing with the study, they have the option to override the quality scoring based on the other quality criteria/considerations. A brief justification is expected to be provided. The scoring is automatically completed based on user responses when the "*Assess Input and Reliability"* button is pressed.

Table 10. Data quality considerations for field TMF data

#	Quality Criterion/Consideration	Maximum Score
1	Field blanks used in the sampling?	30
2	Randomized sampling design employed?	10
3	Biological samples used in B metric co-located and relevant for dietary relationships?	30
4	Biological samples used in B metric obtained in the same year and season?	30
5	Confidence that steady-state is approximated (~±20%)	30
6	Study includes sampling from a minimum trophic level range of 2.0 (i.e., TL 2.0-4.0)	30
7	Method used to derive trophic level provided (e.g., δ15 N /δ13 C stable isotope ratio data available and appropriate baseline organism used.	30
8	Method used to determine the TMF provided?	30
9	Study design incorporates reasonable balance with respect to sample numbers of lower- versus higher-trophic-level organisms?	20
10	Lower trophic level organisms included in sampling (e.g., non-vertebrates)?	20
11	Whole-body analysed? If tissue only analysed, was a correction (normalization) performed?	20
12	Sample concentrations normalized appropriately?	20
13	Analytical standards used in the analysis?	15
14	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)?	15
15	How are measurements below the MDL addressed? Using statistical or replacement methods, or unknown?	15
16	Sampled species names reported?	15
17	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)?	5
18	Environmental temperature reported?	5
19	For ionizables: was pH reported?	5
20	Critical Fail for other reason (override; quality score = 0)	Fail

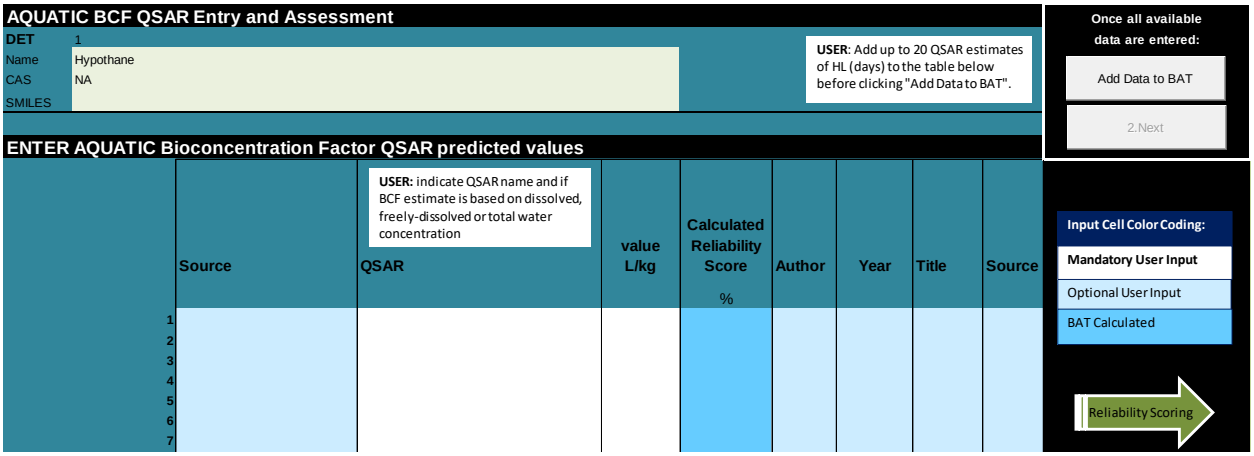
BCF QSARs

The **QSAR-BCF** sheet allows the user to enter predicted BCFs generated by any QSAR model selected by the user. These data are then integrated into the overall assessment conducted by the BAT based on the relevance weighting assigned by the user.

Key Details and Quantitative Information

The input sheet for user-entered BCF QSAR predictions is shown in Figure 27. The mandatory user input for BCF QSAR data includes i) source of the QSAR, ii) name of the QSAR and any relevant details on the output, and iii) the predicted BCF in units of L/kg. Peer-reviewed publication or grey literature regarding the QSAR can also be documented by the user.

Figure 27. Input sheet for user-entered BCF QSAR predictions



Data Reliability Assessment (Data Evaluation Template)

Data quality criteria for in silico predictions of BCF from QSARs are summarized in Table 11. The criteria are based on OECD principles for the validation of QSARs for regulatory purposes [44, 45]. Quality criteria/considerations considered critical are indicated by Pass/Fail. If any one of these quality/criteria/considerations are not met, the QSAR is considered unreliable and the predicted bioaccumulation metric is given a quality score of zero.

In the current version of the BAT, the DET for BCF QSARs must be completed manually be the user. Scroll to the right of the worksheet and enter Y or N as appropriate for each question. The user is responsible for completing the data reliability assessment process for each QSAR prediction entered in the BAT.

Table 11. Data quality considerations for in silico predictions of BCF—QSARs

#	Quality Criterion/Consideration	Maximum Score
1	Is a defined endpoint clearly presented?	Pass/Fail
2	Is the (Q)SAR expressed in the form of a transparent and unambiguous algorithm?	Pass/Fail
3	Is the (Q)SAR associated with appropriate measures of goodness-of-fit, robustness and predictivity?	Pass/Fail
4	Is the (Q)SAR associated with a defined domain of applicability?	15
5	Does the (Q)SAR provide a mechanistic interpretation for the estimate?	15
6	Is the prediction within the stated applicability domain of the QSAR?	30
7	Was the (internal validation) $r^2 > 0.7$?	15
8	Was the (external validation) $q^2_{ext} > 0.5$?	15
9	Critical Fail for other reason (override; quality score = 0)	Fail

In Silico—Bioaccumulation Assessment (BAT)

The BAT conducts an in silico bioaccumulation assessment that includes the following calculations - i) simulation of user-entered laboratory BCF and BMF data, ii) generic simulation of laboratory BCF and BMF tests, and iii) generic simulation of field BAF and BMF for various fish and homeotherms. The generic simulations use a default set of representative conditions characterizing the environmental, biota and food web structure (as required).

Simulation of User-Entered Laboratory BCF and BMF Data

The main purpose of the BAT simulations is to assess the level of agreement (concordance) between the measured laboratory BCF(s) and BMF(s) entered by the user and a standardized model using available data on: i) organism properties (e.g., mass, lipid content), ii) test conditions (e.g., temperature, dissolved oxygen, pH) and exposure scenario (e.g., feeding rate, diet composition) and iii) partitioning properties and susceptibility to biotransformation. Simulations of specific laboratory BCF and BMF tests are only generated by the BAT if sufficient input data about test conditions are provided by the user. Reasonable agreement between the in vivo and in silico BCF and BMF data increases the confidence in the overall B assessment because it indicates that the various LoE are internally consistent. General disagreement between the BAT calculated values and the input data may provide insights into irregularities in the empirical data and/or the model assumptions. **Note that an important consideration and potential cause for discrepancy between the in vivo and in silico BCF and BMF data is the parameterization of the biotransformation rate constant (k_B).** For example, in the absence of in vitro or in silico estimates of biotransformation, the chemical will be assumed to be persistent by the BAT and hence eliminated only via gill ventilation, fecal egestion and growth dilution. The assumption of negligible biotransformation could lead to a substantial underestimation of the total elimination rate constant observed in the study (k_T) and hence a substantial overestimation of the bioaccumulation potential (i.e., BCF or BMF). Careful interpretation of discrepancies between in vivo and in silico BCF and BMF data is required in this situation.

Generic Laboratory BCF and BMF Simulations

In the absence of empirical lab BCF and BMF data entered by the user, the BAT will generate a predicted BCF and BMF based on a generic set of parameters describing the test organism and experimental conditions. The default conditions for the simulations are summarized in Table 12. Additional information about these calculations is presented in the Appendices (A7. In Silico Bioaccumulation Assessment (BAT)).

Generic Field BAF and BMF Calculations

The main purpose of these simulations is to simulate the bioaccumulation of the chemical in a generic aquatic and terrestrial food web using a combined fate and bioaccumulation model built into the BAT [2, 75, 76] to address data gaps, particularly for data poor chemicals. Concentrations in the environment are calculated by the BAT using a Level I fate and transport model (equilibrium partitioning) [77]. Food web bioaccumulation is simulated in each organism assuming steady-state, while disequilibrium resulting from biomagnification or biotransformation or other kinetic factors can occur. Unlike the simulated laboratory BCF and BMF application, no direct comparison is made between available field and in silico data; agreement (concordance) is assessed only with respect to the overall results (i.e., B or not B) arrived at through consideration of multiple LoE.

The organisms included in the generic aquatic and terrestrial food webs are displayed in Figure 28 and Figure 29. Additional information (e.g., body composition, details on feeding relationships) is provided in the Appendices (A7. In Silico Bioaccumulation Assessment).

Table 12. Default input parameters for generic laboratory BCF and BMF simulations (Fish and Rat)

Parameters	Default Value Lab Fish	Default Value Lab Rat
Biological		
Representative Organism	*Generic Lab Fish*	*Wistar rat*
Mass of fish (kg)	0.01	0.25
Storage lipid content of fish	0.043	0.08
Phospholipid content of fish	0.01	0.01
Bulk (structural) protein content of fish	0.147	0.20
Serum albumin of fish	0.003	0.002
Water content of fish	0.797	0.71
Ingestion		
Feeding rate (g food / g fish / d)	0.017	0.054
Storage lipid content of food	0.14	0.04
Phospholipid content of food	0.01	0.01
Bulk (structural) protein content of food	0.45	0.24
Carbohydrate content of food	0.20	0.54
Water content of food	0.20	0.17
Drinking rate (L/h)	-	1.18×10^{-3}
Environmental (laboratory)		
Temperature (°C)	15	-
Bulk water pH	7.0	-
Default dissolved oxygen (mg O_2 / L)	8.56	-
Total organic carbon (TOC; mg/L)	1.5	-

Figure 28. Conceptual representation of the generic aquatic food web included in the BAT in silico

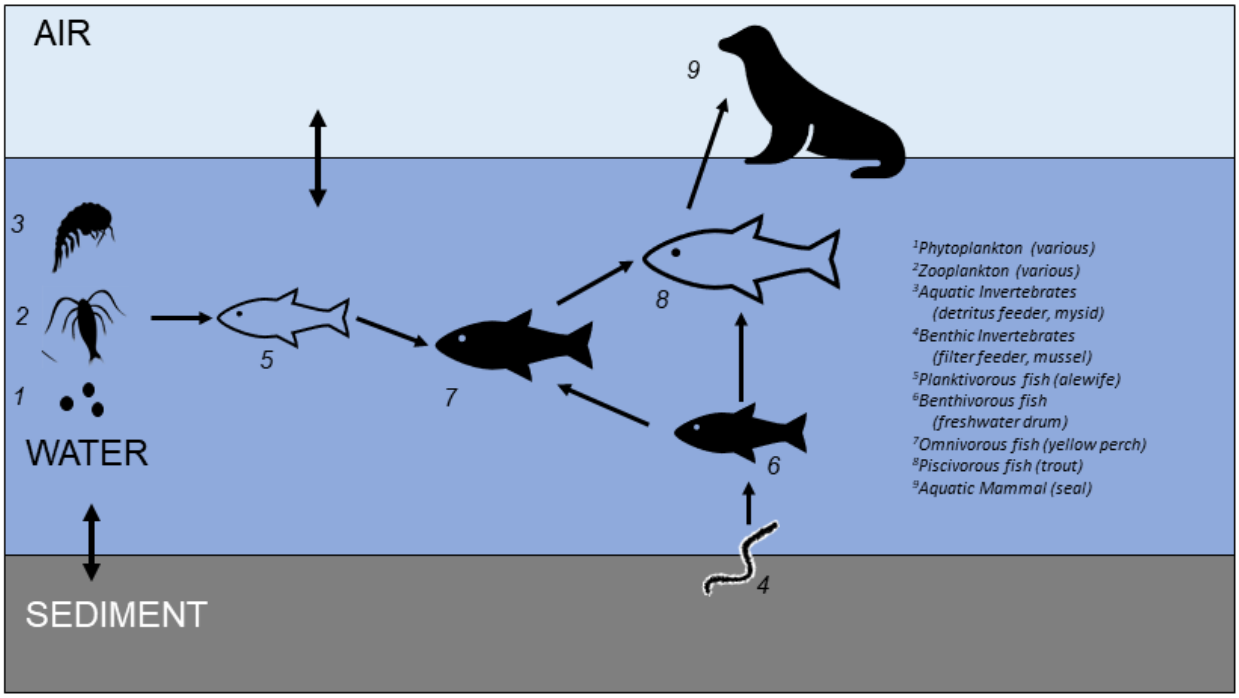
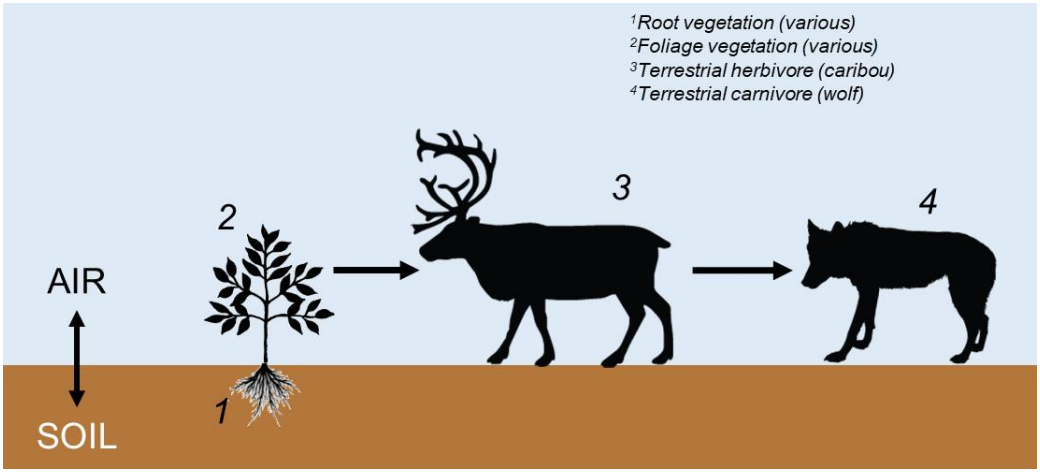


Figure 29. Conceptual representation of the generic terrestrial food web included in the BAT in silico bioaccumulation assessment



Note that the BAT generates in silico field BAFs and BMFs for the aquatic food webs regardless of whether empirical data are available and automatically includes the results from the aquatic food web in the **Results** sheet as described in Table 12. The results from the terrestrial food web can be included if the user selects this option with the "*Do you want to include the BAT in silico output for terrestrial organisms?*" button in the **BAT Main** sheet.

Summary of BAT In Silico Bioaccumulation Assessment

Table 13. Summary of outputs calculated by the default BAT in silico models

LoE	Environment	
BCF	BAT Lab	
	Fish	Kinetic, lipid-standardized (5% lipid content fish) (BCF_L) (growth-corrected or not)
	Invertebrate	Kinetic, wet-weight (BCF)
BAF	BAT Field	
	Low Trophic Level Fish	Steady-state, lipid-standardized (5% lipid content fish) (BAF_L)
	Aquatic Invertebrate	Steady-state, wet-weight (BAF)
	Benthic Invertebrate	Steady-state, wet-weight (BAF)
BMF	BAT Field	
	Upper Trophic Level Fish	Steady-state, lipid-normalized (11% lipid fish, 5.2% lipid diet)
	Seal	Steady-state, lipid-normalized (35% lipid seal, 6.4% lipid diet*)
	Wolf	Steady-state, lipid-normalized (15% lipid wolf, 5.8% lipid diet*)
BMF	BAT Lab	
	Fish	Kinetic, lipid-normalized, (growth-corrected or not) (5.3% lipid fish, 15% lipid diet)
	Rat	Kinetic, lipid-normalized, (growth-corrected or not) (9% lipid rat, 5% lipid diet)

**mass fraction* lipid in diet, including drinking water, e.g. wolf diet lipid ≠ caribou lipid content, although caribou is the sole dietary item (other than drinking water) for wolf.

Fugacity Ratios

Fugacity (*f*, Pa) is an equilibrium criterion calculated from the concentration (*C*, mol/m^3) in a given medium and the sorption (storage) capacity of that medium (*Z*, mol/Pa·m^3), i.e., $f = C/Z$, where *Z* is a function of phase composition (e.g., lipid content, protein content, water content) and partitioning data (e.g., K_{OW}). More information about fugacity and fugacity modelling can be found in Mackay [77]. Fugacity ratios between two phases equal to one indicate that the chemical has achieved thermodynamic equilibrium (i.e., equivalent chemical potential or activity). Fugacity ratios are thus concentration ratios normalized to sorption capacity and in the case of BCF data can also be understood as ratios versus equilibrium partitioning-based values, i.e.,

$$Fugacity\ Ratio = \frac{f_{FISH}}{f_{WATER}} = \frac{C_{FISH}}{C_{WATER}} \cdot \frac{Z_{WATER}}{Z_{FISH}} = \frac{BCF}{K_{FW}}$$

where K_{FW} is the equilibrium fish-water partition coefficient (i.e., Z_{FISH}/Z_{WATER}).

Burkhard et al. [8] outlines an approach to facilitate the interpretation and comparison of laboratory and field B data using fugacity ratios. Using this approach, BAT calculates fugacity ratios for all bioaccumulation data that are internally calculated and that are entered by the user (BCFs, BAFs, BMFs) if required calculation parameters are available. Based on theoretical considerations Mackay [77], BCFs are expected to exhibit fugacity ratios (f_{FISH}/f_{WATER}) equal to or less than one because bioconcentration is driven by organism-water partitioning. Fugacity ratios less than one are often the result of biotransformation occurring in the organism.

Assuming body composition (e.g., lipid content) and partitioning data are accurate, fugacity ratios greater than one for a BCF implies error in the reported concentrations in the organism, water or both.

Fugacity ratios greater than one are possible for BAFs and indicate that dietary uptake is important (i.e., biomagnification is occurring). BMFs with fugacity ratios greater than one also indicate biomagnification whereas BMFs with fugacity ratios less than one indicate biodilution. The extent to which a BAF fugacity ratio exceeds one is a function of chemical and organism properties, food web characteristics, environmental conditions, and trophic level. However, any conclusion drawn from a BAF regarding biomagnification should be consistent with the BMF for the same organism. For example, BAF fugacity ratios much greater than one (biomagnification) cannot be reconciled with BMF fugacity ratios much less than one (biodilution). In other words, fugacity ratios for BMFs and BAFs determined under the same conditions in the same organism should be consistent with respect to exceeding unity or not.

Empirical BCFs with fugacity ratios greater than one are not automatically excluded from the B assessment in the current version of the BAT and discrepancies between BAF and BMF fugacity ratios are not "flagged" for the user either. However, the fugacity ratios provided in the BAT summary output should always be considered as part of the interpretation and decision to include or exclude such data in the WoE.

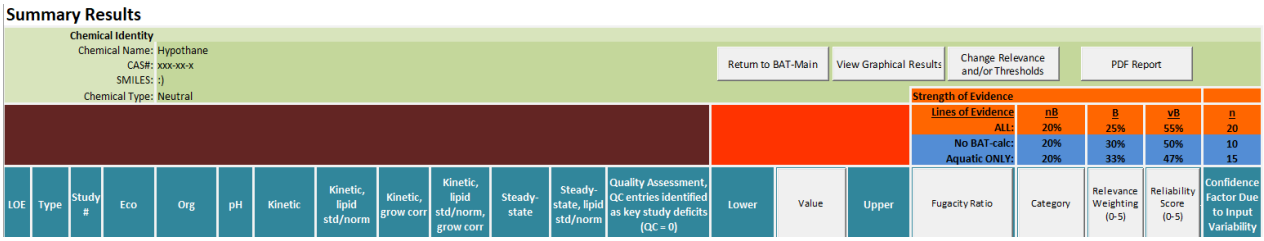
Viewing the Final Results and WoE Summary Information

The results provided by the BAT are summarized on the **Results** worksheet.

Results—Tabled Outputs

A screen capture of the tabular outputs generated by the BAT is provided as Figure 30. Additional details of these outputs are provided below.

Figure 30. Tabled outputs generated by the BAT based on available LoE



LoE

Indicates the bioaccumulation metric being summarized (BCF, BAF, BMF, TMF).

Type

Indicates whether the bioaccumulation metric is from laboratory, field data or in silico-based data.

Study

This number is used with the LoE metric and study type to indicate which sheet the summary results refer to. For example, the first user-entered laboratory BCF is assigned a "1", the second study a "2" etc.

ECO

Indicates whether the LoE is for the aquatic or terrestrial environment.

Org

Indicates type of organism and additional details (e.g., generic lab fish, generic low trophic level fish, wolf or as indicated in entered studies).

pH

Indicates environmental pH of water (aquatic organisms) or pH of water ingested (mammals). This is most relevant for BCFs and BAFs of IOCs.

Kinetic

Output of the wet-weight bioaccumulation metric that is based on a kinetic calculation (e.g., $BCF_K = k_1/k_T$ or $BMF_K = k_D/k_T$)

Kinetic, lipid std/norm

Output of the bioaccumulation metric that is based on a lipid standardized (5% fish) or lipid normalized kinetic calculation, where $fLipid_X$ is fraction lipid of X (e.g., $BCF_{KL} = 0.05/fLipid_{Org} * k_1/k_T$ or $BMF_{KL} = fLipid_{Diet}/fLipid_{Org} * k_D/k_T$)

Kinetic, grow corr

Output of the growth-corrected wet-weight bioaccumulation metric that is based on a kinetic calculation (e.g., $BCF_{KG} = k_1/(k_T-k_G)$ or $BMF_{KG} = k_D/(k_T-k_G)$

Kinetic, lipid std/norm, grow corr

Output of the growth-corrected bioaccumulation metric that is based on a lipid standardized (5% fish) or lipid normalized kinetic calculation, where $fLipid_X$ is fraction lipid of X (e.g., $BCF_{KLG} = 0.05/fLipid_{Org} * k_1//(k_T-k_G)$ or $BMF_{KLG} = fLipid_{Diet}/fLipid_{Org} * k_D/(k_T-k_G)$)

Steady-state

Output of the wet-weight steady-state bioaccumulation metric (e.g., $BCF_{SS} = C_{Biota}/C_{Water}$, $BMF_{SS} = C_{Biota}/C_{Diet}$)

Steady-state, lipid std/norm

Output of the steady-state bioaccumulation metric that is based on lipid standardized or lipid normalized values, where $fLipid_X$ is fraction lipid of X (e.g., $BCF_{SSL} = 0.05/fLipid_{Org} * C_{Org}/C_{Water}$, $BMF_{SSL} = fLipid_{Diet}/fLipid_{Org} * C_{Org}/C_{Diet}$ or TMF)

QC (quality criteria entries identified as key study deficits)

A list of the quality criteria that were not met/fulfilled for a given endpoint based on information provided by the user. The numbers listed correspond to the question number in the DETs. The term “BAT Estimate” is output in this space to indicate the LoE is a BAT model calculation.

Lower, Median Value, Upper

The values of the bioaccumulation metrics selected for assessment are displayed. The output in the **Value** column is based on the reliability score-weighted mean biotransformation rate and E_D; the outputs in the **Lower** and **Upper** columns reflect the approximate range defined by the estimated Confidence Factors (CF) around the reported biotransformation estimates and dietary absorption efficiencies (see **Chemical Summary** sheet). They are not the true minimum and maximum values, but rather indicators of possible range of values based on the uncertainty in the biotransformation rate estimates included in the BAT model simulation.

Fugacity Ratio

The fugacity ratio calculated for each applicable LoE is based on methods detailed in Burkhard et al. [8]. The total sorption capacity of an organism (Z_{BIOTA}) is based on the respective proximate composition (i.e., storage lipid, membrane lipid, serum albumin, storage protein and water content) and the corresponding partition coefficients. If organism lipid contents or lipid-normalized B-metrics are not reported in field or laboratory studies entered into the BAT, a fugacity ratio for those studies cannot be calculated and will appear as a "-".

By definition, the maximum fugacity ratio for a BCF is one, hence BCFs with fugacity ratios greater than one suggest error in the BCF data (C_{FISH} and/or C_{WATER}), proximate composition or partitioning data. Fugacity ratios for BAFs and BMFs can be greater than one and indicate biomagnification. BMFs with fugacity ratios less than one indicate biodilution.

Category

The "B" categorization (nB, B, vB) for a particular LoE based on the output in the **Value** column relative to the **Threshold** selected by the user in the Initialization stage of the BAT. If additional characters appear after these designations, they characterize a change in **categorization** within the minimum to maximum range of predicted estimates. A "*" indicates that the upper predicted boundary value would be assessed at one stage higher than the mean (nB becomes B or B becomes vB). A "**" indicates a categorization change that is two stages higher (nB becomes vB at the high end of predicted values). Similarly, a "-" indicates a shift downwards based on the lower boundary of predicted values (vB to B or B to nB). A "--" indicates that the lower boundary is two categorizations lower than the average (nB from a vB). If the **thresholds** are changed by clicking the "*Change Relevance and/or Thresholds*" button on this sheet, then the categorization for all entered data will be updated.

Relevance Weighting (0-5)

The **Relevance Weighting** (numerical value between 0 – 5) assigned to the bioaccumulation metric (LoE) by the user in the Initialization stage of the BAT. These values can be re-assigned by clicking the "*Change Relevance and/or Thresholds*" button.

Reliability Score (0-5)

The **Reliability Score** of the LoE as determined from the corresponding DET converted from a percentage to a value between 0-5. **Reliability scores are not generated for the BAT calculated (in silico) bioaccumulation metrics**. The in silico BAT calculations are provided in consideration for data-poor chemicals, i.e. to obtain a range of B metrics in the absence of measured data or other estimates, and to provide as comparators if other data are available. Screening-level estimates of uncertainty in BAT calculated values are provided as outlined below, based on variance in user supplied biotransformation rate and dietary absorption efficiency information.

Confidence Factor Due to Input Variability

The values in this column are output for BAT calculated bioaccumulation metrics only. They indicate the confidence factor (CF) surrounding the selected B-metric value based on the variance in entered biotransformation rate and dietary absorption efficiency information. This is the value used to determine the upper (Value * CF) and lower (Value / CF) boundary values output in previous columns.

Strength of Evidence

The **Strength of Evidence** provides a summary (expressed as %) of the bioaccumulation classifications using multiple LoE. A strength of evidence score of 100% occurs if each LoE classification is the same for a particular classification. For example, if all LoE result in a "nB" classification the strength of the evidence for the chemical being "nB" is 100% and the strength of the evidence for the chemical being classified as "B" or "vB" is 0%. Only LoE with Reliability Scores > 0 are considered here. There are three **Strength of Evidence** summaries provided:

1. "ALL" - All LoE input/selected/generated (i.e., BAT-calculated) and user-entered (e.g., lab BCFs, field BAFs, etc)
2. "No BAT-calc." - Only user-entered LoE; no BAT-calculated LoE
3. "Aquatic ONLY", All LoE generated and user-entered except BAT-calculated terrestrial B metrics

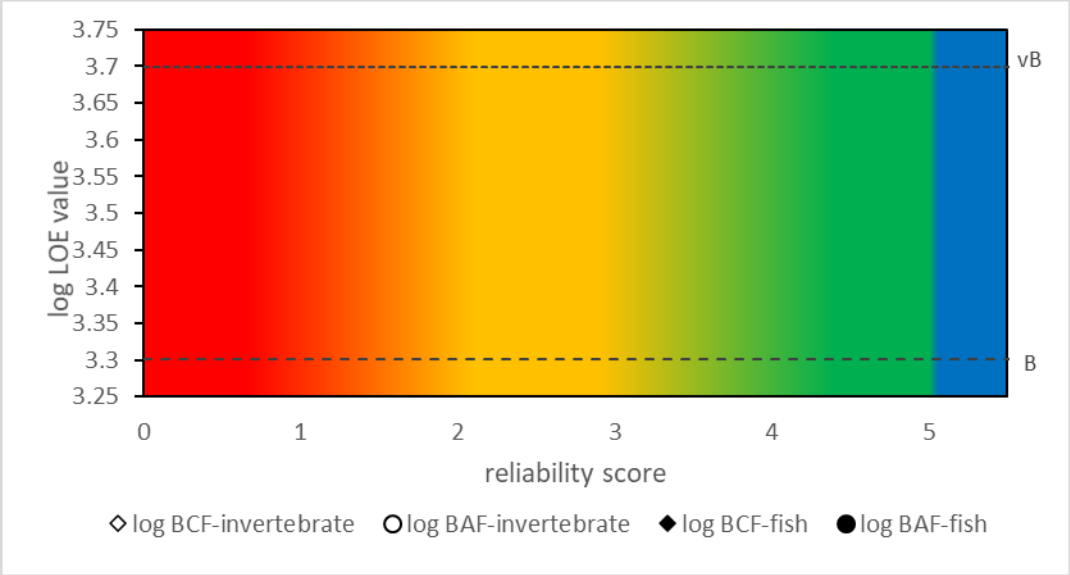
This table is updated if the **threshold** values are changed by clicking the "Change Relevance and/or Thresholds" button.

Results—Graphical Outputs

Classification, Relevance, and Reliability Scores

To facilitate interpretation of the results, the BAT generates figures that can be accessed by clicking on the "*View Graphical Results*" button on the **Results** sheet. Figure 31 provides an example of the general template that summarizes the results. The results are plotted by reliability (0 to 5 on the x-axis) and the numerical value of the LoE (y-axis, scale depends on B metric). Two plots are generated, one for BCFs and BAFs, the other for BMFs and TMFs. The data are displayed by organism types defined (invertebrates, fish, herbivorous homeotherms or carnivorous homeotherms). LoE entered by the user will appear in the red, yellow or green areas of the plot depending on the reliability scores. Data points to the left (in red shaded area) indicate less-reliable LoE and data points on the right (in green shaded area) indicate more reliable LoE. **All LoE calculated by the BAT generic models appear in the blue area as there are no reliability scores associated with these in silico estimates.** This plot allows the user to quickly visualize the results and provides an indication as to the general consistency or lack of consistency in the data. The relative distance between the LoE and the threshold values also highlights the magnitude with which a LoE falls within a particular category.

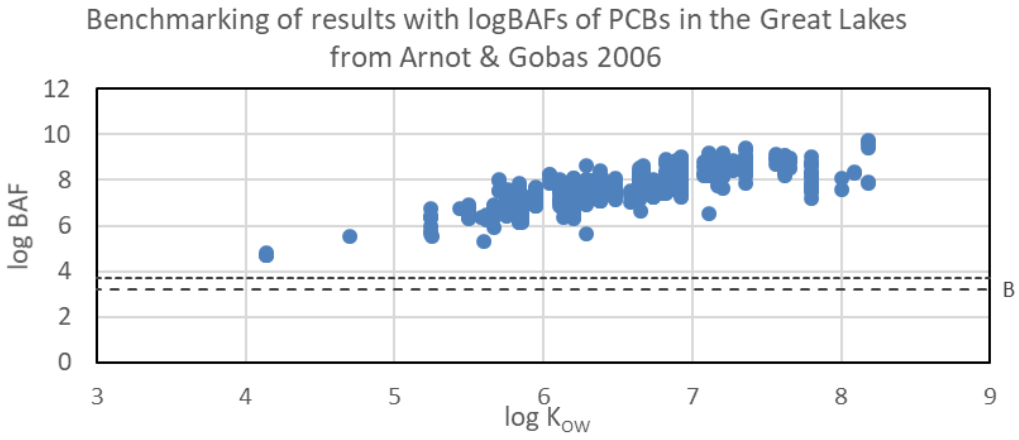
Figure 31. Graphical display of lines of evidence (LoE), e.g., BCFs and BAFs, against reliability scores



Benchmarking

The BAT generates the three figures to benchmark the chemical undergoing evaluation against measured bioaccumulation data for well characterized vB chemicals: polychlorinated biphenyls (PCBs) and hexachlorobenzene (HCB). One figure is based on field BAFs from the North American Great Lakes [3], another figure is based on evaluated laboratory BCFs [3], and the third figures is based on evaluate laboratory measured BMFs [72]. The user-defined thresholds for categorization (e.g., BCF/BAF > 2000, 5000; BMF > 1) are also indicated by labelled horizontal dashed lines. An example of the benchmark data plot for BAFs is shown in Figure 32. Error bars associated with the evaluated chemical reflect the uncertainty (variability) in the biotransformation rate estimates propagated into the BAT calculated B metrics. Lines of Evidence with reliability scores = 0 (Critical Fail) are not included on the benchmarking plots.

Figure 32. Sample benchmarking figure to show where log BAF estimates compare with BAFs of PCBs in the Great Lakes



Source: Arnot and Gobas (2006) [3].

Report PDF

The **Report PDF** collects and summarizes all key inputs and BAT outputs in a format suitable for printing as a PDF. It is generated when the user clicks on the "*Report PDF*" button on the **Results** sheet. Data include information from the **Initialization** sheet including user-defined "B" classification threshold values and relevance weightings, physical-chemical properties, biotransformation rate data, bioaccumulation data, and the graphical output outlined above. The Report PDF mirrors the summary information contained on the **Chemical Summary**, **Results** and **Graphical Results** sheets and provides references to the sheet names that contain each of the study/estimate details. The **Report PDF** also has a colour-coded "status" indicator alongside a numerical indicator for the data presented. If there is an issue with the domain of applicability of the physical-chemical properties, a red "dot" and a "0" appears next to the entry. Likewise, if the reliability score is 0 or a Critical Fail for the biotransformation estimates, then a red dot and a "0" appears beside so the user can quickly distinguish the overall quality of the data, i.e., the presence of red dots flags potential data quality issues. As for the Results, the classification is flagged (vB = red, B = yellow, nB = white), the reliability is flagged (1 or 0 = white, 2 = green, 3 = yellow, 4 = red and 5 = black) and the results are sorted from highest to lowest relevance and flagged (1 or 0 = white, 2 = green, 3 = yellow, 4 = red and 5 = black). A sample of the **Report PDF** can be found in the Appendices (A8. Example of Report PDF Output).

Total elimination half-life, HL_T summary sheet

The **HLT Sheet** summarizes the predicted total elimination half-life for each assessed B-metric, if there are sufficient data to determine it. Each organism in the generic BAT model food webs has various elimination pathways characterized (e.g., respiration, urination, fecal elimination, metabolism, growth), when summed together, they are k_T, the total elimination rate constant (d^{-1}). For laboratory experiments, k_T is often measured from the overall depuration rate of chemical in the organism and the BAT provides space to record this value in each of the Lab BCF (fish and invertebrate) and Lab BMF (fish and rodent) entry sheets. The total elimination half-life, HL_T (d) is calculated as:

$$HL_T = \frac{LN(2)}{k_T}$$

Literature Cited

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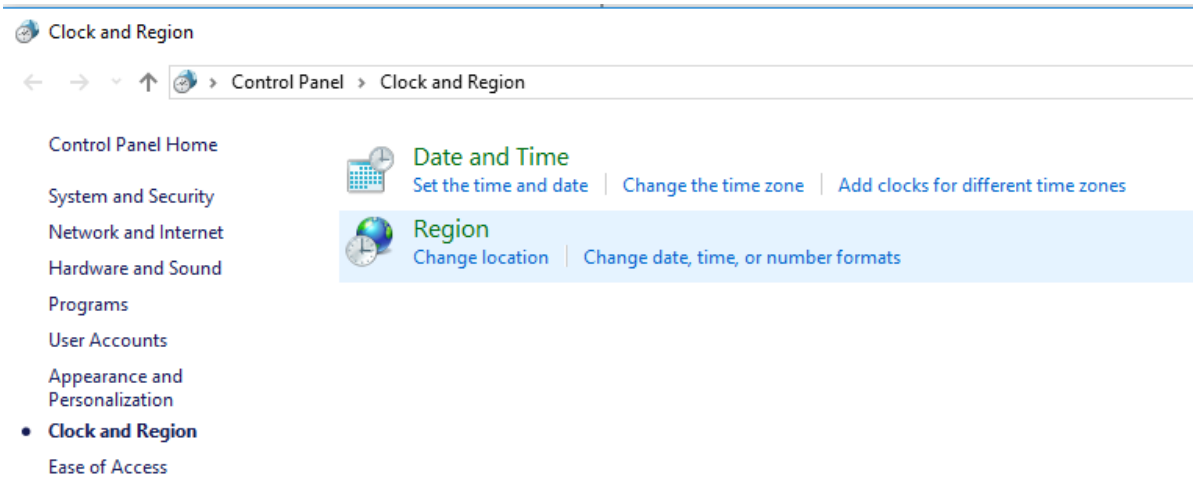
Appendices: Key Equations and Modeling Approaches

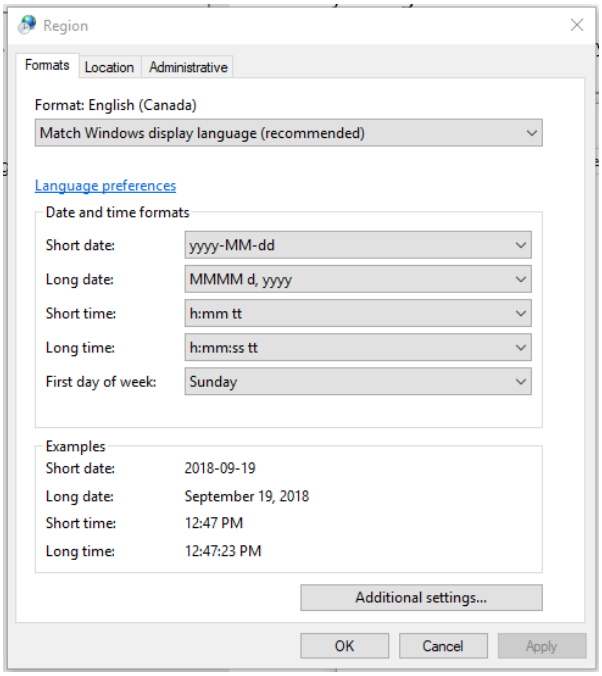
A1. Windows Settings to Ensure Proper Functioning of the BAT

Your computer **must** use the period (.) as the decimal separator rather than the comma (,) to ensure accurate results from the BAT. If your computer is not configured this way, please follow these steps to change the **number formats in Windows 10**:

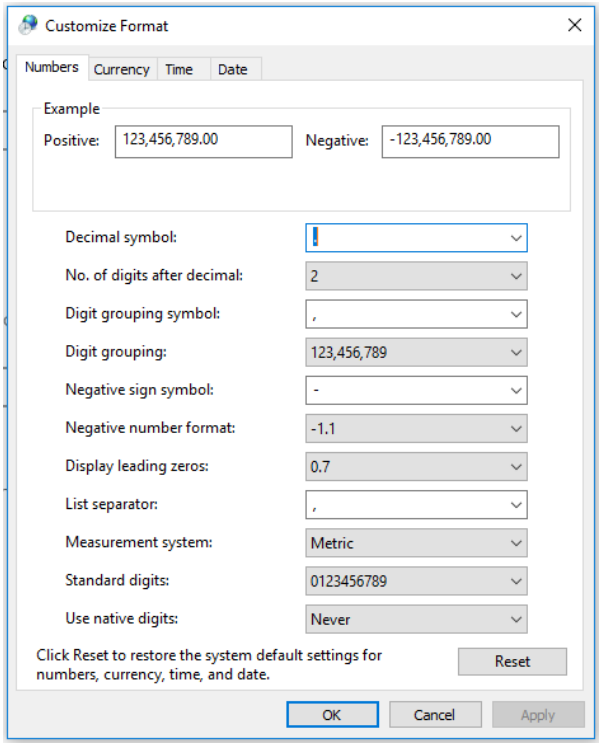
1. Control Panel > Clock and Region (Number formats may be under “Language” or other headings in earlier versions of Windows) > Select **Change number formats**



2. Select “Additional settings…”



3. Select **"."** for the Decimal symbol. It is also recommended to use "," for digit grouping.



A2. Physical-Chemical Properties

Single Parameter Linear Free Energy Relationships (spLFERs)

The spLFERs implemented in the BAT for various partition coefficients are listed below along with the relevant citations.

Particulate organic carbon-water partitioning

$$K_{POC} = 0.35 \cdot K_{OW} \qquad [78]$$

Dissolved organic carbon-water partitioning

$$K_{DOC} = 0.08 \cdot K_{OW} \qquad [79]$$

Storage lipid-water partitioning

$$K_{SW} = K_{OW} \qquad [77]$$

Membrane-water partitioning

$$\log K_{MW} = 1.01 \cdot \log K_{OW} + 0.12 \qquad [80]$$

Structural protein-water partitioning (muscle) and Carbohydrate-water partitioning (plant material)

$$K_{PW} = 0.035 \cdot K_{OW} \qquad K_{CW} = 0.035 \cdot K_{OW} \qquad [81]$$

Bovine serum albumin-water partitioning

If log $K_{OW} \geq 4.5$ [82]

$$\log K_{BSA} = 0.37 \cdot \log K_{OW} + 2.56$$

Else

$$\log K_{BSA} = 1.08 \cdot \log K_{OW} - 0.70$$

Polyparameter Linear Free Energy Relationships (ppLFERs)

The ppLFERs implemented in the BAT were all obtained from the UFZ LSER database (http://www.ufz.de/lserd). Two equations are available for each partition coefficient and are identified following the designation on the website (I or III). Both equations were derived from the same empirical data but Equation I uses the S, A, B, V and L solute descriptors whereas Equation III uses the E, S, A, B and V solute descriptors. For modeling purposes, the averages of the two (log) values are used in the BAT v.1.01.

Octanol-water partitioning

$$\log K_{OW} = -1.41S - 0.18A - 3.45B + 2.41V + 0.43L + 0.34 \qquad \text{I}$$

$$\log K_{OW} = 0.56E - 1.05S + 0.03A - 3.46B + 3.81V + 0.09 \qquad \text{III}$$

where E, S, A, B, V and L are the solute descriptors for the chemical entered by the user.

Dissolved organic carbon-water partitioning

$$\log K_{DOC} = -0.72S + 0.49A - 3.42B + 2.65V + 0.40L - 0.92 \qquad \text{I}$$

$$\log K_{DOC} = 0.29E - 0.52S + 0.63A - 3.40B + 3.94V - 0.85 \qquad \text{III}$$

Storage lipid-water partitioning

$$\log K_{SW} = -1.62S - 1.93A - 4.15B + 1.99V + 0.58L + 0.55 \qquad \text{I}$$

$$\log K_{SW} = 0.70E - 1.08S - 1.72A - 4.14B + 4.11V - 0.07 \qquad \text{III}$$

Membrane-water partitioning

$$\log K_{MW} = -0.93S - 0.18A - 3.75B + 1.73V + 0.49L + 0.53 \qquad \text{I}$$

$$\log K_{MW} = 0.74E - 0.72S + 0.11A - 3.63B + 3.30V + 0.29 \qquad \text{III}$$

Structural protein-water partitioning (muscle), Carbohydrate-water partitioning

$$\log K_{PW} = -0.59S + 0.21A - 3.17B + 2.13V + 0.33L - 0.94 \qquad \text{I}$$

$$\log K_{PW} = 0.51E - 0.51S + 0.26A - 2.98B + 3.01V - 0.65 \qquad \text{III}$$

$$\log K_{CW} = \log K_{PW}$$

Bovine serum albumin-water partitioning

$$\log K_{BSA} = -0.46S + 0.20A - 3.18B + 1.84V + 0.28L + 0.48 \qquad \text{I}$$

$$\log K_{BSA} = 0.36E - 0.26S + 0.37A - 3.23B + 2.82V + 0.27 \qquad \text{III}$$

Default Scaling Factors for Partitioning Properties of Charged vs. Neutral Form (IOCs)

Table A-1. Default scaling factors for partitioning properties of charged vs. neutral form (IOCs)

Partitioning System	Scaling Factor Organic Acid	Scaling Factor Organic Base
Octanol-water	3.16×10^{-4}	3.16×10^{-4}
Suspended solid-water	0.050	1.000
Soil-water	0.050	1.000
Sediment-water	0.050	1.000
Storage lipid-water	0.001	0.001
Phospholipid (membrane)-water	0.100	0.100
Bulk protein-water	0.100	0.100
Serum albumin-water	1.000	0.100

See the following literature sources and references therein for additional information on the default scaling factors for partitioning properties of the charged vs. neutral form (IOCs):

Octanol-water partitioning [31]

Suspended solid-water [83-85]
Soil-water
Sediment-water

Phospholipid-water partitioning [31, 50]
Bulk protein-water partitioning [86]

The selection of default scaling factors for organic acids and bases is challenging because studies where partitioning to serum albumin is studies as a function of pH are not undertaken. However, the general expectation is that organic acids interact more favourably with serum albumin whereas organic bases interact more favourably with other plasma proteins such as alpha 1-acid glycoproteins [87]. Many acidic

pharmaceuticals are predominantly charged at physiological pH yet still exhibit strong affinity for serum albumin. For this reason, we assumed a scaling factor of 1.00 (meaning that in the absence of data, the extent of sorption to albumin is equal to the spLFER or ppLFER for the neutral form of the chemical).

Recently, Bitterman et al. [88] proposed a series of equations for estimating sorption of neutral and ionized (charged) chemicals to muscle and serum albumin using sigma moments as chemical descriptors. The equations were derived from multiple linear regression of available partitioning data using sigma moments calculated with the commercial software COSMOtherm. For neutral organic chemicals (or the neutral form of an IOC), partition coefficients estimated using the sigma moment-based equations can be used in addition to the ppLFER estimates (e.g., by taking an average of the two estimates). For IOCs, partition coefficients estimated using the sigma moment-based equations for the neutral and charged forms can be used to derive distribution ratios (Di,j) at biological pH for bulk protein and serum albumin, which can then be entered into the BAT (see **Figure 10**). With respect to partitioning to phospholipids, Bitterman et al. [88] recommend the use of COSMOmic predictions for the charged form and COSMOmic and/or ppLFER predictions for the neutral form of an IOC. Again, these partition coefficients can be used to derive the distribution ratio (D_{MW}) at biological pH for phospholipids, which can then be entered into the BAT. If the user has access to COSMOtherm and experience using this software, it is recommended that this tool be used rather than relying on the default scaling factors.

A3. In Vitro Biotransformation Rate Data

The general procedure for handling in vitro biotransformation data is the same for liver S9, hepatocyte and liver microsomes and follows general procedures published in the literature, e.g., [61, 64]. OECD guidance documents for these tests can be found on the internet at the URLs listed below:

- **Test No. 319A:** Determination of in vitro intrinsic clearance using cryopreserved rainbow trout hepatocytes (RT-HEP)
  - http://www.oecd.org/publications/test-no-319a-determination-of-in-vitro-intrinsic-clearance-using-cryopreserved-rainbow-trout-hepatocytes-rt-hep-9789264303218-en.htm
- **Test No. 319B:** Determination of in vitro intrinsic clearance using rainbow trout liver S9 sub-cellular fraction (RT-S9)
  - http://www.oecd.org/publications/test-no-319b-determination-of-in-vitro-intrinsic-clearance-using-rainbow-trout-liver-s9-sub-cellular-fraction-rt-s9-9789264303232-en.htm
- **OECD Guidance Document** on the determination of in vitro intrinsic clearance using cryopreserved hepatocytes (RT-HEP) or liver S9 sub-cellular fractions (RT-S9) from rainbow trout and extrapolation to in vivo intrinsic clearance series on testing and assessment.
  - http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=ENV/JM/MONO(2018)12&docLanguage=En

The slope of the depletion curve (log10 concentration vs time in h) entered by the user is converted to a first-order elimination rate constant (and half-life) and then to an intrinsic in vitro clearance ($CL_{IN\ VITRO,\ INT}$) by normalizing the rate constant to the concentration of protein (liver S9, microsome) or cells (hepatocyte) in the test system. In addition to the $CL_{IN\ VITRO,\ INT}$, the BAT estimates the unbound fraction of chemical in the test system (e.g., $f_{U,S9}$) and the ratio of unbound fractions in blood versus the test system (e.g., $f_U = f_{U,B} / f_{U,S9}$). The relevant equations are documented below. The user can also enter their own data for these parameters. In addition to estimating the fraction unbound in assays using QSARs, BAT 2.0 also allows

the user to apply a "compositional approach". QSARs specific to each test system are shown below in the corresponding sections. The compositional approach is described at the end of the section.

First-order rate constant for biotransformation in test system (1/h)

$$k_E = -2.3 \cdot slope$$

where *slope* is the slope of the depletion curve (log10 concentration vs time in h) calculated from the in vitro test data by linear regression and entered by the user.

First-order half-life for biotransformation in test system (h)

$$HL = \frac{\ln(2)}{k_E}$$

Liver S9

Intrinsic in vitro clearance (ml/h/mg S9)

$$CL_{INVITRO,INT} = \frac{k_E}{C_{S9}}$$

where C_{S9} is the reported concentration of S9 protein in the test medium (mg S9/ml) and $CL_{IN\ VITRO,\ INT}$ is in units of ml/h/mg S9

Unbound fraction in in vitro system (neutral organic chemicals) "BAT Estimated"

$$f_{U,S9} = \frac{1}{C_{S9} \cdot 10^{0.694 \cdot \log K_{OW} - 2.158} + 1.0} \qquad [61]$$

Austin et al. [89] published a regression-based equation for predicting the unbound fraction of chemicals in microsomal in vitro systems based on empirical data for neutral organic chemicals and IOCs. Predictions are made using the octanol-water partition coefficient for neutral and basic IOCs (i.e., log K_{OW} and log $K_{OW,N}$ respectively) and the octanol-water distribution ratio at pH 7.4 (D_{OW}) for acidic IOCs. Lacking a similar analysis for the S9 test system, the same approach is adopted here, i.e.,

Unbound fraction in in vitro system (acidic IOCs)

$$f_{U,S9} = \frac{1}{C_{S9} \cdot 10^{0.694 \cdot \log D_{OW,pH\,7.4} - 2.158} + 1.0}$$

Unbound fraction in in vitro system (basic IOCs)

$$f_{U,S9} = \frac{1}{C_{S9} \cdot 10^{0.694 \cdot \log K_{OW,N} - 2.158} + 1.0}$$

Unbound fraction in in vitro system "Compositional"

*see below

Hepatocyte

$$CL_{INVITRO,INT} = \frac{k_E}{C_{HEP}}$$

where C_{HEP} is the concentration of liver cells in the test medium (10^6 cells/ml) and $CL_{IN\ VITRO,\ INT}$ is in units of ml/h/10^6 cells.

Unbound fraction in in vitro system (neutral organic chemicals) "BAT Estimated"

$$f_{U,HEP} = \frac{1}{(C_{HEP} / 2 \cdot 10^6) \cdot 10^{0.676 \log K_{OW} - 2.215} + 1.0} \qquad [61]$$

Austin et al. [89] published a regression-based equation for predicting the unbound fraction of chemicals in microsomal in vitro systems based on empirical data for neutral organic chemicals and IOCs. Predictions are made using the octanol-water partition coefficient for neutral and basic IOCs (i.e., log K_{OW} and log $K_{OW,N}$ respectively) and the octanol-water distribution ratio at pH 7.4 (D_{OW}) for acidic IOCs. Lacking a similar analysis for the HEP test system, the same approach is adopted here, i.e.,

Unbound fraction in in vitro system (acidic IOCs)

$$f_{U,HEP} = \frac{1}{(C_{HEP} / 2 \cdot 10^6) \cdot 10^{0.676 \log D_{OW,pH7.4} - 2.215} + 1.0}$$

Unbound fraction in in vitro system (basic IOCs)

$$f_{U,HEP} = \frac{1}{(C_{HEP} / 2 \cdot 10^6) \cdot 10^{0.676 \log K_{OW,N} - 2.215} + 1.0}$$

Unbound fraction in in vitro system "Compositional"

*see below

Microsome

$$CL_{INVITRO,INT} = \frac{k_E}{C_{MIC}}$$

where C_{MIC} is the concentration of microsomal proteins in the test medium (mg/ml) and $CL_{IN\ VITRO,\ INT}$ is in units of ml/h/mg protein.

Unbound fraction in in vitro system (neutral organic chemicals) "BAT Estimated"

$$f_{U,MIC} = \frac{1}{C_{MIC} \cdot 10^{0.56 \log K_{OW} - 1.41} + 1.0} \qquad [89]$$

Unbound fraction in in vitro system (acidic IOCs)

$$f_{U,MIC} = \frac{1}{C_{MIC} \cdot 10^{0.56 \log D_{OW,pH7.4} - 1.41} + 1.0} \qquad [89]$$

Unbound fraction in in vitro system (basic IOCs)

$$f_{U,MIC} = \frac{1}{C_{MIC} \cdot 10^{0.56 \log K_{OW,N} - 1.41} + 1.0} \qquad [89]$$

Unbound fraction in in vitro system "Compositional"

*see below

Compositional Approach to estimate fraction unbound in in vitro assays

The compositional approach to estimate fractions unbound in in vitro assays is based on the composition of the assay (storage lipid, membrane lipid, protein, and water content) and partitioning data. The fraction unbound in the assay ($f_{U,\,assay}$) is estimated as shown below.

$$f_{U,assay} = \frac{f_W}{K_{assay-w}}$$

where f_W is the water content of the assay and $K_{assay\text{-}w}$ is the assay-water partition coefficient (or distribution ratio for IOCs). The assay-water partition coefficient (or distribution ratio for IOCs) is calculated using the following expression:

$$K_{assay-w} = f_{SL}K_{SW} + f_{PL}K_{MW} + f_{BP}K_{PW} + f_W$$

where f_{SL},f_{PL} and f_{BP} are the storage lipid, phospholipid and protein content of the assay and K_{SW}, K_{MW} and K_{PW} are the storage lipid water, membrane water and protein water partition coefficients respectively.

For S9 and MIC assay systems, the protein content is based on the user-entered concentration of protein (mg/ml) and the storage lipid and membrane lipid contents are calculated using assumed lipid:protein ratios. The water content of the assay is calculated as the remainder. The default storage lipid-protein and membrane lipid-protein ratios are 0 and 0.35 respectively [65, 66].

The estimated composition of a HEP assay system is based on i) the volume of individual hepatocyte cells (3.4×10^{-12} L/cell), the composition of each cell and the number of cells/ml assay entered by the user. The default hepatocyte cell composition is 0.02, 0.045, 0.19 and 0.745 for storage lipids, phospholipids, protein and water respectively. Assay compositions for example S9, MIC and HEP systems are summarized in Table A-2.

Table A-2. Assay compositions for sample S9, MIC and HEP systems

Assay System	Protein (mg/ml) or Hepatocyte concentration (10^6 cell/ml)	Phase	Fraction
S9 or MIC	2	Storage lipid	0
		Phospholipid	0.0007
		Protein	0.002
		Water	0.9973
HEP	1	Storage lipid	6.75×10^{-5}
		Phospholipid	1.52×10^{-4}
		Protein	6.41×10^{-4}
		Water	9.99×10^{-1}

A4. In Vitro-In Vivo Extrapolation (IVIVE)

As described previously, the steps required for IVIVE 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 whole-body biotransformation rate constant (k_B). The IVIVE calculations require information on physiology, liver protein and cell contents, body and blood composition and partitioning, as summarized in Table A-3, Table A-4 and Table A-5 for fish, rats, and humans respectively [58, 61, 64, 68, 90].

Liver S9

$$CL_{INVIVO,INT} = CL_{INVITRO,INT} \cdot PL \cdot LW$$

where PL is the S9 protein content of the liver (mg S9 / g liver) and LW is the liver weight as a fraction of the total body weight (g liver / g body weight).

Hepatocyte

$$CL_{INVIVO,INT} = CL_{INVITRO,INT} \cdot CL \cdot LW$$

where CL is the cellularity of the liver (10^6 cells / g liver) and LW is the liver weight as a fraction of the total body weight (g liver / g body weight).

Microsome

$$CL_{INVIVO,INT} = CL_{INVITRO,INT} \cdot PL \cdot LW$$

where PL is the microsomal protein content of the liver (mg protein / g liver) and LW is the liver weight as a fraction of the total body weight (g liver / g body weight).

Table A-3. IVIVE parameters and default values for fish in vitro data

Parameter	BAT Symbol	Units	Default Values
Liver weight (fraction of total BW)	LW	g liver / g BW	0.015
Protein content of liver (S9, MIC)	PL	mg protein / g liver	163, 40
# of liver cells/g liver (HEP)	CL	10^6 cells / g liver	500
Total cardiac output	QC	L/h/kg (ml/h/g)	*Calc.* [91]
Fraction of cardiac output to liver	LF	-	0.259
Organism			
Total lipid content*	$f_{L,B}$	-	0.05
Bulk protein content*	$f_{BP,B}$	-	0.15
Water content	$f_{W,B}$	-	0.80
Blood			
Total lipid content*	$f_{L,Bl}$	-	0.014
Bulk protein content	$f_{BP,Bl}$	-	0.1225
Serum albumin content	$f_{SA,Bl}$	-	0.0225
Water content	$f_{W,Bl}$	-	0.841
Blood-water partition coefficient	P_{BW}	L/L	*Calc.*
Biota-water partition coefficient	BCF_P	L/kg (ml/g)	*Calc.*
Volume of distribution	$V_{D,\ Bl}$	L/kg (ml/g)	*Calc.*
Fraction unbound in whole blood	$f_{U,Bl}$	-	*Calc.*
Ratio of fraction unbound	f_U	-	*Calc.*
Intrinsic clearance, in vivo	$CL_{in\ vivo,int}$	ml/h/g BW	*Calc.*
Hepatic clearance	CL_H	L/h/kg (ml/h/g)	*Calc.*
Whole-body biotransformation rate constant	k_B	h^{-1}	*Calc.*

*Total lipids in organism assumed to be 1% phospholipids (membrane), remainder storage lipids; total lipids in blood divided equally between phospholipids (membrane) and storage lipids [92]; Whole-body fraction of albumin =0.003, remainder = bulk protein

Table A-4. IVIVE parameters and default values for rat in vitro data

Parameter	BAT Symbol	Units	Default Values
Liver weight (fraction of total BW)	LW	g liver / g BW	0.038
Protein content of liver (S9, MIC)	PL	mg protein / g liver	143, 23.4
# of liver cells/g liver (HEP)	CL	10^6 cells / g liver	120
Total cardiac output	QC	L/h/kg (ml/h/g)	*Calc.* [93]
Fraction of cardiac output to liver	LF	-	0.183
Organism			
Total lipid content*	$f_{L,B}$	-	0.05
Bulk protein content*	$f_{BP,B}$	-	0.23
Water content	$f_{W,B}$	-	0.72
Blood			
Total lipid content*	$f_{L,Bl}$	-	0.0033
Bulk protein content	$f_{BP,Bl}$	-	0.1342
Serum albumin content	$f_{SA,Bl}$	-	0.0225
Water content	$f_{W,Bl}$	-	0.84
Blood-water partition coefficient	P_{BlW}	L/L	*Calc.*
Biota-water partition coefficient	BCF_P	L/kg (ml/g)	*Calc.*
Volume of distribution	$V_{D,\ Bl}$	L/kg (ml/g)	*Calc.*
Fraction unbound in whole blood	$f_{U,Bl}$	-	*Calc.*
Ratio of fraction unbound	f_U	-	*Calc.*
Intrinsic clearance, in vivo	$CL_{in\ vivo,int}$	ml/h/g BW	*Calc.*
Hepatic clearance	CL_H	L/h/kg (ml/h/g)	*Calc.*
Whole-body biotransformation rate constant	k_B	h^{-1}	*Calc.*

*Total lipids in organism assumed to be 1% phospholipids (membrane), remainder storage lipids; total lipids in blood divided equally between phospholipids (membrane) and storage lipids [92]; Whole-body fraction of albumin =0.003, remainder = bulk protein

Table A-5. IVIVE parameters and default values for human in vitro data

Parameter	BAT Symbol	Units	Default Values
Liver weight (fraction of total BW)	LW	g liver / g BW	0.0257
Protein content of liver (S9, MIC)	PL	mg protein / g liver	143, 32
# of liver cells/g liver (HEP)	CL	10^6 cells / g liver	100
Total cardiac output	QC	L/h/kg (ml/h/g)	*Calc.* [93]
Fraction of cardiac output to liver	LF	-	0.227
Organism			
Total lipid content*	$f_{L,B}$	-	0.20
Bulk protein content*	$f_{BP,B}$	-	0.20
Water content	$f_{W,B}$	-	0.60
Blood			
Total lipid content*	$f_{L,Bl}$	-	0.0033
Bulk protein content	$f_{BP,Bl}$	-	0.1342
Serum albumin content	$f_{SA,Bl}$	-	0.0225
Water content	$f_{W,Bl}$	-	0.84
Blood-water partition coefficient	P_{BlW}	L/L	*Calc.*
Biota-water partition coefficient	BCF_P	L/kg (ml/g)	*Calc.*
Volume of distribution	$V_{D,\ Bl}$	L/kg (ml/g)	*Calc.*
Fraction unbound in whole blood	$f_{U,Bl}$	-	*Calc.*
Ratio of fraction unbound	f_U	-	*Calc.*
Intrinsic clearance, in vivo	$CL_{in\ vivo,int}$	ml/h/g BW	*Calc.*
Hepatic clearance	CL_H	L/h/kg (ml/h/g)	*Calc.*
Whole-body biotransformation rate constant	k_B	h^{-1}	*Calc.*

* Total lipids in organism assumed to be 1% phospholipids (membrane), remainder storage lipids; total lipids in blood divided equally between phospholipids (membrane) and storage lipids [92]; Whole-body fraction of albumin =0.003, remainder = bulk protein

Hepatic clearance (CL_H)

$$CL_H = \frac{Q_H \cdot f_U \cdot CL_{INVIVO,INT} \cdot \frac{f_{W,assay}}{f_{W,Bl}}}{Q_H + f_U \cdot CL_{INVIVO,INT} \cdot \frac{f_{W,assay}}{f_{W,Bl}}}$$

where Q_H is the amount of blood flowing to the liver (ml/h/g), f_U is the ratio of the unbound fractions in blood and the in vitro test system. Following Krause and Goss [94], the ratio of water contents in the assay and blood is also included as a term in the CL_H expression

$$f_U = \frac{f_{U,Bl}}{f_{U,i}}$$

$$f_{U,Bl} = \frac{f_{W,Bl}}{P_{BlW}}$$

where $f_{W,Bl}$ is the volume fraction of water in blood and P_{BlW} is the blood-water partition coefficient.

Nichols et al. (2013) [61] recommend the following equation for P_{BlW}, which is taken from Fitzsimmons et al. (2001) [67].

$$P_{BlW} = (10^{0.73 \cdot \log K_{OW}} \cdot 0.16) + 0.84$$

where 0.16 represents the bulk organic matter content and 0.84 is the water content of blood.

While deemed suitable for neutral organic chemicals, the use of a bulk organic matter term is problematic for IOCs because of the much larger differences in sorption affinities between storage lipids, phospholipids, bulk protein, and serum albumin exhibited by these chemicals. An option to calculated P_{BlW} using more detailed information on phase composition and the corresponding partition coefficients is therefore provided. The equation for neutral organic chemicals is shown below:

$$P_{BlW} = f_{SL,Bl} K_{SW} + f_{PL,Bl} K_{MW} + f_{BP,Bl} K_{PW} + f_{SA,Bl} K_{BSA} + f_{W,Bl}$$

where $f_{SL,Bl}$ is the fraction of storage lipids in whole blood, $f_{PL,Bl}$ is the fraction of phospholipids in whole blood, $f_{BP,Bl}$ is the fraction of bulk protein in whole blood (excluding albumin), $f_{SA,Bl}$ is the fraction of serum albumin in whole blood, $f_{W,Bl}$ is the fraction of water in whole blood and K_{SW} is the storage lipid-water partition coefficient, K_{MW} is the membrane-water partition coefficient, K_{PW} is the bulk (structural) protein-water partition coefficient, and K_{BSA} is the bovine serum albumin-water partition coefficient. For IOCs, the various partition coefficients are replaced by the corresponding distribution ratios (e.g., D_{MW} instead of K_{MW}). Note that this approach to calculate P_{BlW} is consistent with Nichols et al. (2006) [64].

The whole-body biotransformation rate constant (k_B) is calculated from hepatic clearance (CL_H) by dividing this parameter by the estimated Volume of Distribution (referenced to blood), $V_{D,Bl}$.

$$k_B = \frac{CL_H}{V_{D,Bl}}$$

$V_{D,Bl}$ is estimated as shown below

$$V_{D,Bl} = \frac{BCF_P}{P_{BlW}}$$

where BCF_P is the equilibrium partition coefficient between the organism and water (i.e., K_{BW}). BCF_P is estimated using partitioning data and whole-body composition, as shown below:

$$BCF_P = K_{BW} = f_{SL,B}K_{SW} + f_{PL,B}K_{MW} + f_{BP,B}K_{PW} + f_{SA,B}K_{BSA} + f_{W,B}$$

where $f_{SL,B}$ is the whole-body fraction of storage lipids, $f_{PL,B}$ is the whole-body fraction of phospholipids, $f_{BP,B}$ is the whole-body fraction of bulk (structural) protein, $f_{SA,B}$ is the whole-body fraction of serum albumin, $f_{W,B}$ is the whole-body fraction of water and K_{SW} is the storage lipid-water partition coefficient, K_{MW} is the membrane-water partition coefficient, K_{PW} is the bulk (structural) protein-water partition coefficient, and K_{BSA} is the bovine serum albumin-water partition coefficient. For IOCs, the various partition coefficients are replaced by the corresponding distribution ratios (e.g., D_{MW} instead of K_{MW}).

A5. Calculation of Average Biotransformation Rate and Dietary Absorption Efficiencies

The BAT uses a robust assessment of data to determine a single biotransformation rate constant and/or dietary absorption efficiency for each organism type. The process by which this is calculated is the same for entered biotransformation rates and for dietary absorption efficiencies, although the weighting assigned to all E_D entries is 1 (Wt = 1). For simplicity, the process is described below for biotransformation rates, however $k_{B,Ni}$ could be replaced with $E_{D,Ni}$ for the calculation of $E_{D,N,AVG}$.

Each time an estimate for a biotransformation rate is entered for either fish or mammals, a weighted average of the estimates for each organism class is calculated and output on the Physical-Chemical output sheet. The average k_B for fish or mammals is calculated as:

$$k_{B,N,AVG} = \exp\left(\frac{\sum\left(\ln k_{B,Ni} * Wt * Rel_i\right)}{\sum Wt * Rel_i}\right)$$

where $k_{B,N,i}$ and Rel_i are the body mass normalized whole-body biotransformation rate constant and relevance scoring (0-1) from i Line of Evidence (e.g., in vitro or in silico or in vivo) and Wt is the weighting assigned to each biotransformation LoE method (Table A-6). These values appear on the Chemical Summary sheet next to the biotransformation output summary and may be changed by the user. Entering a value of "0" will exclude biotransformation rate constants of that source from the calculation of $k_{B,N,AVG}$ for each organism.

Table A-6. Default weighting scores associated with biotransformation rate study types

Biotransformation Line of Evidence (study type)	Weighting (*Wt*)
In Vivo	1
In Vitro	0.7
In Silico (trained on In Vivo datasets)	0.8
In Silico (trained on In Vitro datasets)	0.6

There is one $k_{B,N,AVG}$ for fish based on $k_{B,Ni}$ for fish and one $k_{B,N,AVG}$ for mammals based on $k_{B,Ni}$ for mammals and a $k_{B,N}$ for invertebrates based on the scaling factors selected. The reliability (confidence) score Rel_i for

each $k_{B,Ni}$ and the selected Wt from Table A-6 is used to weight the average value, such that higher reliability score data and In Vivo and In Vivo-based LoE have a greater influence on the averages used in the BAT model calculations. Based on reviews we have conducted for thousands of reliability scores for in vitro biotransformation rate data and typical reliability scores obtained for the in silico biotransformation rate predictions during BAT development and testing, the default weighting scores for in vitro estimates are set at 0.7. In silico estimates derived from in vivo datasets are assigned a weighting score of 0.8 and in silico estimates trained on in vitro datasets are assigned a weighting score of 0.6 and these values are used as "default" weighting. Confidence Factors (CF) of $k_{B,N,AVG}$ are also calculated in the BAT model as per Slob 1994 [71] to characterize the variance in model output based on the variance in model input.

A6. Bioaccumulation Data (Empirical)

Laboratory BCF

The BAT calculates the following BCF metrics based on information provided by the user, i) steady-state, wet-weight BCF, standardized to 5% lipid content fish ($BCF_{SS,L}$), ii) kinetic wet-weight BCF (BCF_K), iii) growth-corrected kinetic BCF ($BCF_{K,G}$), iv) kinetic, wet-weight, BCF standardized to 5% lipid content fish ($BCF_{K,L}$), and v) growth-corrected, wet-weight, kinetic BCF, standardized to 5% lipid content fish ($BCF_{K,L,G}$) [4]. The various calculations are listed below.

$$BCF_{SS,L} = BCF_{SS} \cdot \frac{0.05}{f_L}$$

$$BCF_K = \frac{k_1}{k_T}$$

$$BCF_{K,G} = \frac{k_1}{(k_T - k_G)}$$

$$BCF_{K,L} = BCF_K \cdot \frac{0.05}{f_L}$$

$$BCF_{K,L,G} = BCF_{K,G} \frac{0.05}{f_L}$$

where BCF_{SS} is the steady-state bioconcentration factor entered by the user, f_L is the (average) total lipid content of the test organisms, k_1 is the gill uptake rate constant (L/kg/d), k_T is the total elimination rate constant (/d) and k_G is the rate constant (/d) for growth dilution.

Laboratory BMF

The BAT calculates the following BMF metrics based on information provided by the user, i) kinetic BMF (BMF_K), ii) growth-corrected kinetic BMF ($BMF_{K,G}$), iii) lipid-normalized kinetic BMF ($BMF_{K,L}$), and iv) growth-corrected and lipid-normalized BMF ($BMF_{K,L,G}$) [4, 95].

$$BMF_K = \frac{I \cdot E_D}{k_T} = \frac{k_D}{k_T}$$

$$BMF_{K,G} = \frac{I \cdot E_D}{(k_T - k_G)} = \frac{k_D}{(k_T - k_G)}$$

where I is the food consumption rate (g food/g fish/d), E_D is the chemical uptake efficiency across the gut, also denoted as α [95], k_T is the total elimination rate constant and k_G is the rate constant for growth dilution. If no E_D data are entered, E_D (or α) is calculated using the following expression:

$$E_D = \alpha = \frac{C_{0,B} \cdot k_T}{I \cdot C_D \cdot (1 - e^{-k_T \cdot t})}$$

where $C_{0,B}$ is the (average) concentration of the chemical in the organism at the beginning of the depuration phase (mg/kg), C_D is the (average) concentration of the chemical in the diet (mg/kg) and t is the duration of the uptake phase (d).

$$BMF_{K,L} = BMF_K \cdot \frac{f_{L,D}}{f_{L,B}}$$

$$BMF_{K,L,G} = BMF_{K,G} \cdot \frac{f_{L,D}}{f_{L,B}}$$

where $f_{L,D}$ is the average lipid content of the ingested food and $f_{L,B}$ is the average lipid content of the test organism over the experiment.

Additional BMF-Related Metrics Calculated by the BAT

Body-gut partition coefficient (K_{BG})

$$K_{BG} = \frac{C_B}{C_G} = \frac{Z_B}{Z_G} = \frac{f_{L,B} + \phi \cdot f_{P,B} + f_{W.B} / K_{OW}}{f_{L,G} + \phi \cdot f_{P,G} + f_{W,G} / K_{OW}}$$

where C_B and C_G are the concentrations of the chemical in the organism and gut at equilibrium, Z_B and Z_G are the relative sorption capacities of the organism and gut and the lipid (L), protein (P) and water contents (W) of the diet and gut are represented by the corresponding $f_{i,j}$ terms in the equation. The ϕ term is a proportionality constant relating the sorption capacity of lipid to protein and has a default value of 0.035 [81]. The composition of the gut contents is a function of the composition of the diet and the gut absorption efficiencies entered by the user or the default values (see Laboratory BMF DET).

Diet-gut partition coefficient (K_{DG})

$$K_{DG} = \frac{C_D}{C_G} = \frac{Z_D}{Z_G} = \frac{f_{L,D} + \phi \cdot f_{P,D} + f_{W.D} / K_{OW}}{f_{L,G} + \phi \cdot f_{P,G} + f_{W,G} / K_{OW}}$$

where C_D and C_G are the concentrations of the chemical in the diet and gut at equilibrium, Z_D and Z_G are the relative sorption capacities of the diet and gut and the lipid (L), protein (P) and water contents (W) of the diet and gut are represented by the corresponding $f_{i,j}$ terms in the equation.

Theoretical maximum BMF excluding biotransformation (BMF_{MAX}) [73]:

$$BMF_{MAX} = \frac{G_D Z_D}{\frac{k_G Z_B}{E_D} + G_F Z_F}$$

where G_D and G_F are the feeding and fecal egestion rates (based on ingestion rate entered by user and gut absorption efficiencies) and k_G is the growth dilution rate constant (entered by user or calculated by the BAT).

The BMF_{MAX} is the expected wet weight BMF based on the gastrointestinal magnification caused by the reduction in volume (G_D vs G_F) and sorption capacity (Z_D vs Z_G) that occurs during the digestion process. Growth dilution is accounted for, but biotransformation is not. Accordingly, laboratory BMFs substantially lower than the BMF_{MAX} indicate biotransformation and/or reduced absorption are important factors mitigating the bioaccumulation potential of the chemical whereas laboratory BMFs approaching the BMF_{MAX} indicate that the chemical is readily absorbed and persistent.

A7. In Silico Bioaccumulation Assessment (BAT)

Biota-Water Partitioning

The BAT calculates various bioaccumulation metrics using information entered by the user (e.g., physical-chemical property data, biotransformation data) and parameters representing environmental conditions (e.g., temperature, pH) and characteristics of biota (e.g., mass, body composition, feeding relationships) using one-compartment toxicokinetic models. The rate constants for all chemical uptake and elimination processes, including biotransformation are assumed to be first-order and passive (no active transport processes are considered). A key component of all model calculations is the estimated total sorption capacities of the biota, which are represented by biota-water partition coefficients for neutral organic chemicals and biota-water distribution ratios for IOCs. The general equation for a biota-water partition coefficient (K_{BW}) is as follows:

$$K_{BW} = f_{SL} K_{SW} + f_{PL} K_{MW} + f_{BP} K_{PW} + f_{SA} K_{BSA} + f_W$$

where f_{SL} is the whole-body fraction of storage lipids, f_{PL} is the whole-body fraction of phospholipids, f_{BP} is the whole-body fraction of bulk (structural) protein, f_{SA} is the whole-body fraction of serum albumin, f_W is the whole-body fraction of water and K_{SW} is the storage lipid-water partition coefficient, K_{MW} is the membrane-water partition coefficient, K_{PW} is the bulk (structural) protein-water partition coefficient, and K_{BSA} is the bovine serum albumin-water partition coefficient. For IOCs, the various partition coefficients are replaced by the corresponding distribution ratios (e.g., D_{MW} instead of K_{MW}) to arrive at the biota-water distribution ratio (D_{BW}).

Generic Modeling Approaches for Aquatic Organisms

The methods for estimating gill uptake and the various elimination rate constants for neutral organic chemicals in fish are fully described in Arnot and Gobas [2] and Arnot et al. [17, 18]. The approaches used in BAT Ver.2.0 have evolved the treatment of the gill uptake and diet absorption efficiencies for fish and have now included the original approaches from Arnot and Gobas and Arnot et al. for invertebrates.

In brief, the gill uptake rate constant for fish and invertebrates (k_1; L/kg/d) is based on an estimated gill ventilation rate (G_V, L/d) normalized to organism mass (kg) and the chemical uptake efficiency at the gill

(E_W). Gill ventilation is estimated as a function of oxygen demand (M, mg/d) according to the following equations, the second of which relates M to fish size (mass in kg, W) and temperature (T, ºC) [17] as:

$$G_V = \frac{M}{C_{OX} E_{OX}}$$

$$\log M = 2.80 + 0.786 \log W + 0.017T$$

where C_{OX} is the concentration of dissolved oxygen in the water column (mg/L) and E_{OX} is the absorption efficiency of oxygen across the gills (default value = 0.65).

For aquatic invertebrates and zooplankton, the gill uptake efficiency, E_W parameter for neutral chemicals is estimated from K_{OW} using the following equation:

$$E_W = \left(1.85 + \frac{155}{K_{OW}}\right)^{-1} + \beta$$

where β is an assumed baseline (minimum) uptake efficiency with a default value of 0.01. Armitage et al. [31] extended the mechanistic bioaccumulation model from Arnot and Gobas [2] for neutral organic chemicals to ionizable organic chemicals (IOCs).

For fish, E_W is calculated following the approach developed in the ECO21 project (ECO21-ARC: Improving the performance and expanding the applicability of a mechanistic bioconcentration model for ionogenic organic compounds (IOCs) in fish (BIONIC) – Cefic-Lri (cefic-lri.org).

$E_W = \beta_{EW} + 1/(1+a * (b + c))$

Gill elimination is a function of the gill uptake rate constant and partitioning between the organism and water (K_{BW} or D_{BW}). Fecal egestion is based on the food ingestion rate (G_D or I, g food/g fish/d), chemical uptake efficiency in the gut (E_D), gut absorption efficiencies, and partitioning between the body and egesta (K_{BG}, see previous section).

Table A-7. Modified approach to calculation of the E_W parameter for fish

Parameter	Value	Parameter	Equation
R_W	1.85	a	$=G_V$ * Z (GILL, LIQUID)
R_L	155	b	$=1/ (G_V / (R_W-1)$ * Z(GILL, LIQUID))
β_{EW}	0.0001	Neutrals: c	$=1 / (G_V$ / RL * Z(GILL, SOLID))
γ_{EWacid}	10000	Ionics: c	$=1 / (G_V$ / RL* Z_N(GILL, SOLID) * (f_N(GILL, LIQUID)) + G_V / RL / γ_{EW}* Z_I(GILL, LIQUID) * f_I(GILL, LIQUID))
γ_{EWbase}	300000		
Other:			
GV, fI, fN	Gill Ventilation Rate (m^3/h), fraction ionized at gill pH, fraction neutral at gill pH		

Z_T(GILL, LIQUID), (Z_N, Z_I)	Fugacity capacity of water fraction of gill material at gill pH (total, neutral and ionic Zs)
Z_T(GILL, SOLID) , (Z_N, Z_I)	Fugacity capacity of solid fraction of gill material at gill pH (total,neutral)

Bioenergetic balance in Fish

Following a bioenergetically-based bioaccumulation model for fish by Quinn et al. [96], the food ingestion rate for aquatic organisms is generated in two stages. First, the allometric relationship for the estimation of the food ingestion rate (G_D) in kg/d for is estimated as a function of body size (W in kg) and temperature (T in °C) as:

$$G_D = 0.022 \cdot W^{0.85} \cdot \exp(0.06 \cdot T)$$

and then normalized to organism size (i.e., converted to units of g food / g fish / d).

This value of G_D is used to parameterize the growth rate expected in aquatic organisms using the allometric equation from [97].

$$k_G = 0.00586 \cdot 1.113^{(T-20)} \cdot W^{-0.2}$$

The growth rates predicted in this manner have been compared to literature values. The growth rate forms the basis of the bioenergetic needs (caloric intake from diet and respiration rate) of the aquatic organism.

The Invertebrate E_D parameter for neutral chemicals is estimated from K_{OW} using the following equation:

$$E_D = \left(a + b \cdot K_{OW}\right)^{-1}$$

where the default values in BAT for *a* and *b* for invertebrates are 2.0 and 5 x10^{-8} respectively [1, 98]. Based on the review by Abraham et al. [99], the chemical uptake efficiency in the gut for IOCs was estimated from octanol-water partitioning of the neutral form without accounting for speciation. See Armitage et al. [31] for additional discussion of this topic.

The fish E_D parameter used for neutral chemicals is preferentially read in as the weighted average of user-entered empirical fish E_D values. The parameters required for this calculation are hard-coded and are found in Table A-8.

Table A-8. Dietary absorption efficiency parameters for all higher-level organism types

Parameter	Fish	Herbivores	Omnivores/Carnivores
GOct	1.19x10^{-10}	6.5x10^{-5}	3x10^{-5}
GW	0.02119	1000	950
VOct	3.37x10^{-9}	0.001	6x10^{-6}
VW	1.11x10^{-7}	0.0001	1x10^{-5}

Gut transport HL, τ_{trans} (h)	20	30	8
Gut reaction HL, τ_{rxn} (h)	$1x10^{12}$	$1x10^{12}$	$1x10^{12}$

The calculation consists of two stages. First the half-lives of chemical in the gut (T_G) and chemical crossing the gut-body barrier (T_A) are calculated.

$$\tau_G = \frac{1}{\left(\frac{1}{\tau_{rxn}} + \frac{1}{\tau_{trans}}\right)}$$

$$\tau_A = VW + VOct * Kow * \left(\frac{1}{GOct * Kow} + \frac{1}{Gw}\right)$$

Then use this information to calculate E_D:

$$E_D = 1 - \exp\left(-\frac{\tau_G}{\tau_A}\right)$$

A minimum E_D value of 0.001 has been selected as a conservative approach in BAT.

The biotransformation rate constant (k_B) is based on the various lines of evidence pertaining to this process entered by the user (e.g., in silico, in vivo and in vitro).

Generic Modeling Approaches for Terrestrial/Air-Breathing Organisms

The conceptual approach for modeling bioaccumulation in terrestrial organisms is very similar to how aquatic organisms are handled, e.g., [10, 12, 13]. Exposure can occur via inhalation, food ingestion and drinking water whereas elimination can occur via i) exhalation, ii) fecal egestion, iii) urinary excretion, iv) biotransformation and v) growth dilution.

The basic equation for estimating steady-state concentrations in terrestrial organisms (i.e., function of rate constants and concentrations in exposure media) matches the equations for aquatic organisms. However, as BCFs and BAFs are referenced to the water phase, such metrics are not typically considered for terrestrial organisms. BMFs and TMFs are commonly calculated though. Unlike fish, homeotherms (i.e., mammals) are at different temperatures than their surrounding environment and temperature can influence chemical partitioning. For each chemical, BAT adjusts the chemical partitioning information entered by the user (assumed to be ~ 25 °C) to their corresponding values at 37 °C according to the methods outlined by McLeod et al. [100].

Exposure in the BAT generic terrestrial organisms is calculated using flow rates (G, m^3/d air, food, drinking water) and concentrations of the chemical in the respective media. The general equation for estimating the flow rates for terrestrial organisms is shown below:

$$G_i = [a \cdot M^b] \cdot Act$$

where *a* and *b* are allometric scaling factors, *M* is the body size of the organism (kg) and *Act* is a multiplier to account for energy expenditure in the field.

Default values of these parameters for the terrestrial herbivore and carnivore including in the BAT model are reported in Table A-9.

Table A-9. Allometric scaling factors used to estimated flow rates in terrestrial organisms

Organism and Exposure Media	a	b	Act
Terrestrial herbivore (caribou)			
Inhalation (air)	0.550	0.800	1.5
Food	0.080	0.73	4.0
Drinking water	0.099	0.900	1.0
Terrestrial carnivore (wolf)			
Inhalation (air)	0.550	0.800	1.5
Food	0.045	0.820	4.0
Drinking water	0.099	0.900	1.0
Aquatic Mammal (seal)			
Inhalation (air)	3.60	0.75	1.0
Food	0.069	0.822	4.0
Drinking water	0.000	0.900	1.0
Lab Rat (Wistar Rat)			
Inhalation (air)	0.550	0.800	1.0
Food	*assumed 0.054 g food/g rat/day		
Drinking water	0.099	0.900	1.0

Biotransformation rate constants (k_B) for terrestrial organisms are based on user-input and scaled for size.

The growth rates of the caribou and wolf are assumed to be $0.002 \cdot G_D$ and $0.0005 \cdot G_D$, where G_D is the flow rate of food (m^3/d) calculated using the values in Table A-9. The growth rate constant (k_G, 1/d) is calculated by dividing the growth rate by M (in m^3).

As for fish, in the absence of user-input data, E_D for the different classes of mammals is calculated using the parameters defined in Table A-8 and the equations that follow it.

Laboratory BCF

Laboratory BCFs are calculated for the in silico bioaccumulation assessment using a 1-compartment toxicokinetic modelling approach [2, 17, 18, 31]. Exposure is assumed to occur only via respiration (gill ventilation) and the main equation is presented below:

$$BCF = \frac{C_B}{C_W} = \frac{k_1 \cdot C_W}{C_W \cdot k_T} = \frac{k_1}{k_T}$$

where C_B and C_W are the concentrations in the organism and water respectively, k_1 is the gill uptake rate constant (L/kg/d) and k_T is the total elimination rate constant (1/d).

The total elimination rate constant is the sum of the following individual elimination pathways, i) gill elimination (k_2), ii) fecal egestion (k_E), iii) biotransformation (k_B), and iv) growth dilution (k_G). Fecal egestion is included as a loss process because it is assumed that the organisms are being fed clean food throughout the exposure and depuration periods. For simulation of the user-entered empirical BCF study, rate constants are based on the data provided by the user with missing data estimated using the generic

approaches described above. For the generic simulation of a laboratory BCF, all inputs except the food ingestion rate (G_D or I, g food / g fish / d) are estimated values. Note that the default food ingestion rate is 0.02 g food/g fish/d (see Table 12).

Laboratory BMF

Laboratory BMFs are calculated for the in silico bioaccumulation assessment using a 1-compartment toxicokinetic modelling approach [2, 17, 18, 31]. Exposure is assumed to occur only via dietary exposure and the main equation is presented below:

$$BMF = \frac{C_B}{C_D} = \frac{k_D C_D}{C_D \cdot k_T} = \frac{k_D}{k_T}$$

The dietary uptake rate constant (k_D) is a function of the food ingestion rate (G_D or I, g food/g fish/d) and chemical uptake efficiency in the gut (E_D) (see previous section). For simulation of the user-entered empirical BMF study, rate constants are based on the data provided by the user with missing data estimated using the generic approaches described above. For the generic simulation of a laboratory BMF, all inputs except the food ingestion rate (G_D or I, g food / g fish / d) are estimated values. Note that the default food ingestion rate is 0.02 g food/g fish/d (see Table 10).

Field BAF and BMF

Field BAFs for fish are calculated for the in silico bioaccumulation assessment using a 1-compartment toxicokinetic modelling approach [2, 17, 18, 31]. Exposure is assumed to occur via respiration (gill ventilation) and food ingestion (dietary uptake) and the main equation is presented below:

$$BAF = \frac{C_B}{C_W} = \frac{k_1 \cdot C_W + k_D C_D}{C_W \cdot k_T}$$

where C_B, C_W and C_D are the concentrations in the organism, water, and diet respectively, k_1 is the gill uptake rate constant (L/kg/d), k_D (kg/kg/d) is the dietary uptake rate constant and k_T is the total elimination rate constant (1/d). The total elimination rate constant is the sum of the following individual elimination pathways, i) gill elimination (k_2), ii) fecal egestion (k_E), iii) biotransformation (k_B), and iv) growth dilution (k_G). All rate constants are estimated using the generic approaches described above.

The equation for field BMF for fish is like the BAF equation except that the predicted concentration in the organism is divided by the predicted concentration in its diet, i.e.,

$$BMF = \frac{C_B}{C_D} = \frac{k_1 \cdot C_W + k_D C_D}{C_D \cdot k_T}$$

where C_B, C_W and C_D are the concentrations in the organism, water, and diet respectively, k_1 is the gill uptake rate constant (L/kg/d), k_D (kg/kg/d) is the dietary uptake rate constant and k_T is the total elimination rate constant (1/d). In all of the BAT calculated metrics, C_W is the freely-dissolved (bioavailable) chemical concentration in the water as C_W (or more explicitly, C_{WD}) = ϕC_{WT}.

For terrestrial organisms, field BMF is calculated accounting for exposure via inhalation and drinking water in addition to dietary uptake, i.e.,

$$BMF = \frac{C_B}{C_D} = \frac{k_A \cdot C_A + k_D C_D + k_W C_W}{C_D \cdot k_T}$$

If dietary uptake strongly dominates (as expected for many hydrophobic chemicals), the BMF calculation can be simplified to BMF = k_D / k_T.

Key Aquatic Organisms and Feeding Relationships

Field

Table A-10. Body size and composition of organisms in the generic aquatic food web

Organism	Mass (kg)	SL	PL	BP	BCn	SA	W
Phytoplankton	$1x10^{-11}$	0.005	0.005	0	0.06	0	0.93
Zooplankton	$3x10^{-7}$	0.015	0.005	0.10	0	0	0.88
Benthic invertebrate	$2.4x10^{-3}$	0.015	0.005	0.10	0	0	0.88
Aquatic invertebrate	$2x10^{-5}$	0.025	0.005	0.12	0	0	0.85
Planktivorous fish	0.05	0.04	0.01	0.147	0	0.003	0.80
Benthivorous fish	0.1	0.04	0.01	0.147	0	0.003	0.80
Omnivorous fish	0.3	0.06	0.01	0.147	0	0.003	0.78
Piscivorous fish	2.5	0.10	0.01	0.147	0	0.003	0.74
Seal	60	0.39	0.01	0.16	0	0.003	0.4375
Sediment/Detritus	-	0	0	0	1	0	0

SL = storage lipid, PL = phospholipid (membrane), BP = bulk protein, BCn = bulk carbon SA = serum albumin, W = water

Table A-11. Feeding habits of the aquatic organisms in the generic aquatic food web

Organism	Diet
Phytoplankton	-
Zooplankton	100% phytoplankton
Benthic invertebrate	40% phytoplankton, 50% zooplankton, 10% sediment/detritus
Aquatic invertebrate	10% phytoplankton, 70% zooplankton, 20% sediment/detritus
Planktivorous fish	5% phytoplankton, 55% zooplankton, 10% benthic invertebrate, 30% aquatic invertebrate
Benthivorous fish	25% zooplankton, 10% benthic invertebrate, 30% aquatic invertebrate, 5% sediment/detritus

Omnivorous fish	30% zooplankton, 20% benthic invertebrate, 20% aquatic invertebrate, 20% planktivorous fish, 10% benthivorous fish
Piscivorous fish	10% zooplankton, 40% planktivorous fish, 25% benthivorous fish, 25% omnivorous fish
Seal	5% benthic invertebrates; 10% planktivorous fish, 10% benthivorous fish, 75% omnivorous fish

Laboratory

Table A-12. Body size and composition of organisms in the generic laboratory aquatic food web.

Organism	Mass (kg)	SL	PL	BP	BC	SA	W
Lab fish	0.01	0.043	0.01	0.147	0	0.003	0.797
Lab invertebrate	$1x10^{-5}$	0.015	0.005	0.15	0	0	0.83
Fish feed	-	0.14	0.01	0.45	0.20	0	0.20
Invertebrate feed	-	0.11	0.01	0.46	0.36	0	0.06

SL = storage lipid, PL = phospholipid (membrane), BP = bulk protein, BC = bulk carbohydrate SA = serum albumin, W = water

BAT-modelled generic laboratory fish consume fish feed at the rate of 0.017 g food/g fish/day or $7.19x10^{-9}$ m^3/h. Laboratory invertebrates consume the invertebrate feed at a rate of $9.39x10^{-11}$ m^3/h. For BCF estimations, the feed is set as "clean" (fugacity and concentration = 0) and uptake is only via respiration of contaminated water. For BMF studies, the water is set as "clean" and uptake is only via ingestion of contaminated diet.

Key Terrestrial Organisms and Feeding Relationships

Table A-13. Body size and composition of organisms in generic terrestrial food web

Organism	Mass (kg)	SL	PL	BP	BC	SA	W
Foliage vegetation	-	0.0056	0.0038	0.0521	0.1043	0	0.8342
Root vegetation	-	0.0054	0.0036	0.05	0.1	0	0.8410
Terrestrial herbivore	120	0.09	0.01	0.197	0	0.003	0.70
Terrestrial carnivore	90	0.14	0.01	0.147	0	0.003	0.70

SL = storage lipid, PL = phospholipid (membrane), BP = bulk protein, BC = bulk carbohydrate, SA = serum albumin, W = water

Table A-14. Feeding habits, including proportion of diet that is drinking water of the terrestrial organisms in the generic terrestrial food web. Values in brackets represent proportion of diet, not considering drinking water.

Organism	Diet
Foliage vegetation	-
Root vegetation	-
Terrestrial herbivore	57 (95)% foliage vegetation, 3 (5)% root vegetation, 40% drinking water
Terrestrial carnivore	58 (100)% terrestrial herbivore, 42 (0)% drinking water

Table A-15. Body size and composition of organisms in the generic laboratory aquatic food web.

Organism	Mass (kg)	SL	PL	BP	BC	SA	W
Lab rat	0.25	0.080	0.010	0.20	0	0.002	0.708
Mammal feed	-	0.040	0.010	0.240	0.540	0	0.170

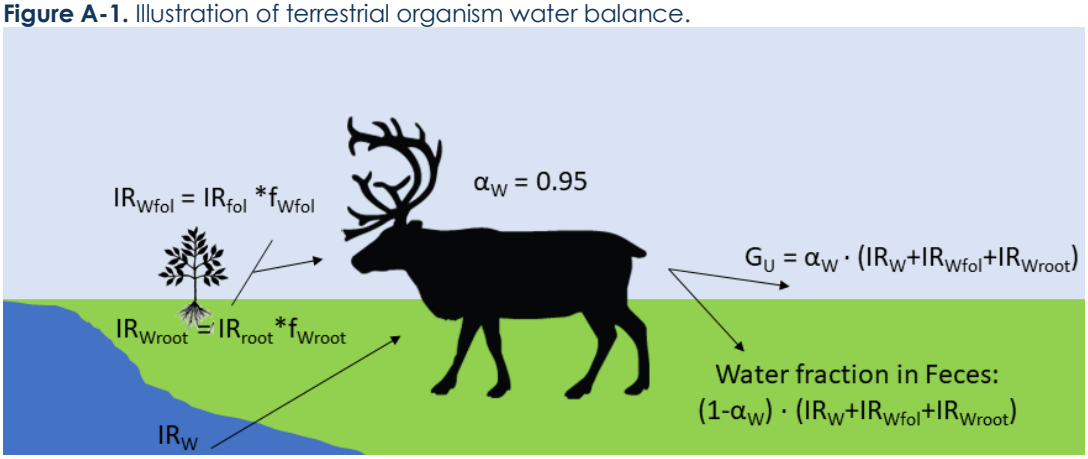
SL = storage lipid, PL = phospholipid (membrane), BP = bulk protein, BC = bulk carbohydrate, SA = serum albumin, W = water

The Generic BAT lab rat feeds at a rate of 0.054 g food/g rat/day or 5.65×10^{-7} m^3/h. It is assumed to consume only "clean" water, so a drinking intake is not explicity included, however, the urinary outflow is considered as if the rat is drinking "clean" water at a the allometrically set rate of 1.18×10^{-6} m^3/h. The lab rat also considers only intake of "clean" air. Uptake of chemical is only via contaminated diet.

Mammalian Water Balance

Mammals in BATver.2.0 have an internal water balance; drinking rate, water intake via the diet, absorption efficiency of water into the system and excretion of water via urine and feces is considered. Figure A-1 illustrates the method by which the water balance for is treated for mammals. It shows how the rate of intake of *water,* (IR_{WX} m_3/h) from each ingested item is quantified. A defined water absorption efficiency (α_W) is applied to calculate how much of the diet item is absorbed into the body and subsequently excreted as urine (G_U, m^3/h) and how much is passed through into the feces (1- α_W). No other losses of water via respiration, for example, are considered.

Figure A-1. Illustration of terrestrial organism water balance.



A8. Example of Report PDF Output

The Bioaccumulation Assessment Tool (BAT) Ver.2.02
developed by ARC Inc. with support from CEFIC-LRI
Prepared by: ARC-2
Organization: ARC
Report created on: 2021-08-31 10:28

Bioaccumulation Assessment Report

Project Summary

BAT v2.02 **CAS #: v2.02** **Neutral**

SMILES:

Summary Scenario Line of Evidence	B Threshold	vB Threshold	Relevance Weight
Laboratory Fish BCF	2000	5000	3
Laboratory Invertebrate BCF	2000	[illegible]	3
Field BAF	2000	[illegible]	3
In Silico BCF	2000	[illegible]000	3
In Silico BAF	2000	5000	3
Laboratory BMF	1	1	3
Laboratory Mammal TK-BMF	1	1	3
Field BMF	1	1	3
In Silico Aq BMF	1	1	3
In Silico Terr BMF	1	1	3
Field TMF	1	1	3

Status:

Chemical Summary

Hexachlorobenzene **118-74-1** **Neutral**

	User Entered	spLFER/ ppLFER	Used in BAT	Status:
Molecular Weight (g/mol)	300.00		300.00	3
Water Solubility (mg/L)	1.00E+00		1.00E+00	
Vapor Pressure (Pa)		8.25E-03	8.25E-03	
Henry's Law Constant ($Pa/m^3 \cdot mol$)		2.48E+00	2.[illegible]E+00	
$logK_{AW}$	-3.00		[illegible]	
$logK_{OW}$	5.00		5.00	3
$logK_{OA}$		[illegible]	8.00	3
$logK_{POC}$		[illegible]54	4.54	
$logK_{DOC}$		3.90	3.90	
$logK_{StorageLipidW}$			5.19	3
$logK_{MembraneLipidW}$		5.50	5.50	3
$log_{ProteinW}$			3.73	3
$logK_{CarbohydrateW}$			3.73	
$logK_{SerumAlbuminW}$		4.74	4.74	
Solubility in Octanol (mol/m^3)		333.00	333.00	3

BAT v2.02, CAS #: v2.02

Reliability

Biotransformation Estimate Summary

	hours	days	CF	n
Standardized Half-life in Fish (10g, 15°C)				
Standardized Half-life in Mammals (70kg)				
Standardized Half-life in Invert (10ug, 15°C)				
Standardized Half-life in Autotrophs/Plants (1kg)				

** denotes critical fail in biotrans study, value NOT used

Biotransformation summary	0.01 kg FISH k_B	70 kg MAMMAL kB	0.01mg INVERT k_B	1 kg AUTOTROPH/PLANT	Reliability Score
k_B					
		Fish	Herbivore	Carnivore	

Dietary absorption efficiency (%)
Confidence Factor

Dietary absorption efficiency summa	0.01 kg FISH E_D	HERBIVORE E_D	CARNIVORE E_D	Reliability Score

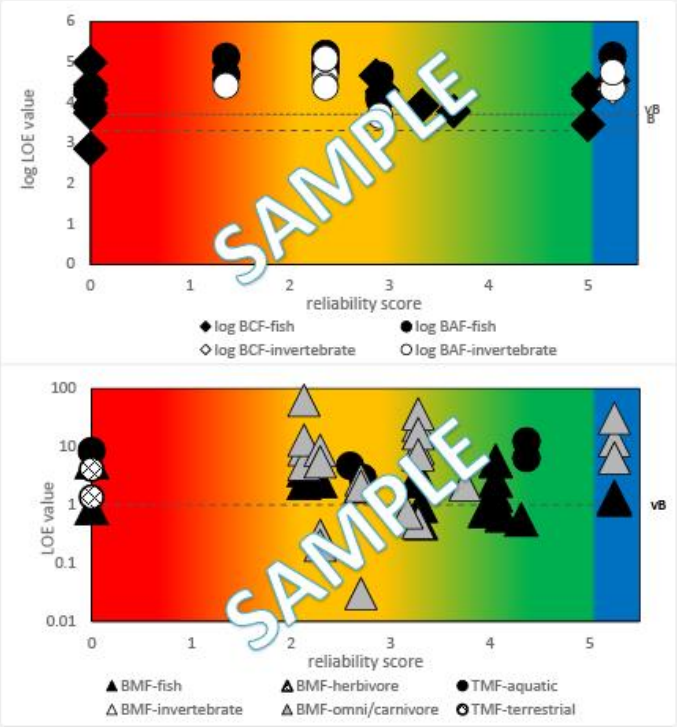
BAT v2.02, CAS #: v2.02

	Strength of Evidence:	nB:	B:	vB>1		Reliability 1,2,3,4,5	
	ALL:	15.58%	2.60%	vB>1			
	No BAT-calc:	14.93%	2.99%	vB>1			
	All Aquatic:	10.91%	3.64%	vB>1	4		3
Bioaccumulation Estimate Summary				vB>5000	4		3

B-metric	LOE Source	Sheet Ref.	Value	vB>5000	4		3
BAF: Aquatic-Fish-BAT Generic Field Low TL Fish	BAT Estimate	N/A	1.57E+04	vB>5000	4		3
BAF: Aquat. Invert.-BAT Generic Field Aquat. Invert.	BAT Estimate	N/A	7.97E+03	nB≤2000	2		3
BAF: Aquat. Invert.-BAT Generic Field Benthic Invert.	BAT Estimate	N/A	6.00E+03	vB>5000	4		3
BMF: Aquatic-Fish-BAT Generic Field Upper TL Fish	BAT Estimate	N/A	0.958	vB>5000	4		3
BMF: Homeotherm-Omni./Carn.-BAT Generic Field Seal	BAT Estimate	N/A	25.6	vB>5000	4		3
BMF: Homeotherm-Omni./Carn.-BAT Generic Field Wolf	BAT Estimate	N/A	75.6	vB>5000	4		3
BMF: Homeotherm-Omni./Carn.-BAT Generic Lab Rat	BAT Estimate	N/A	9.5	vB>5000	4		3
BMF: Aquatic-Fish-BAT Generic Lab Fish	BAT Estimate	N/A	0.303	vB>5000	4		3
BCF: Aquatic-Fish-BAT Generic Lab Fish	BAT Estimate	N/A	1.04E+04	vB>5000	4		3
BCF: Aquat. Invert.-BAT Generic Lab Invert	BAT Estimate	N/A	5.04E+03	vB>5000	4		3

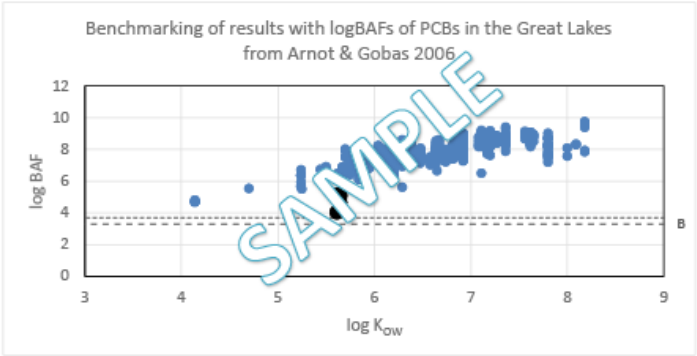
BAT v2.02, CAS #: v2.02

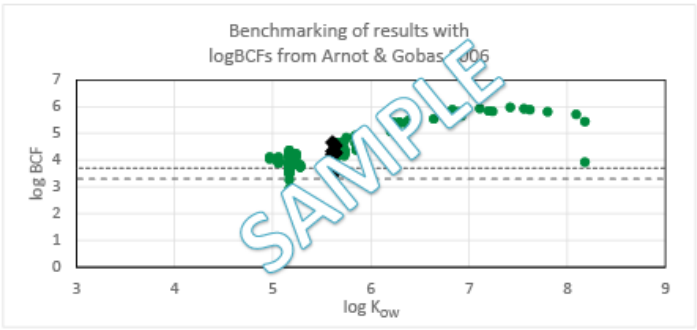
Benchmarking Figures

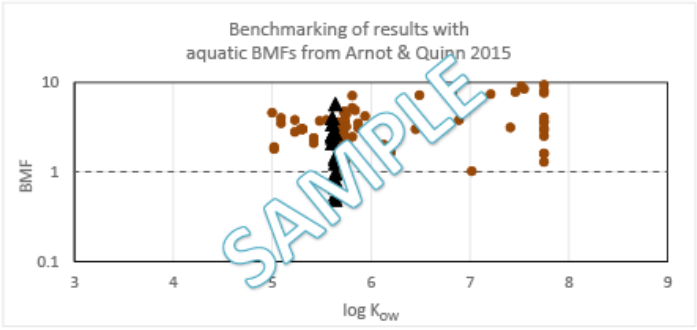


BAT v2.02, CAS #: v2.02

Benchmarking Figures







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References

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