

The Bioaccumulation Assessment Tool (BAT)

Version 2.1

QUICK START GUIDE

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Arnot Research & Consulting

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QUICK START GUIDE

Using the Bioaccumulation Assessment Tool (BAT)

Updates in Ver.2.1 are listed at the end of this document

The BAT will only function properly on 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 the User Manual. This quick start user guidance can be considered to expedite the use of the BAT; however, all BAT users are strongly encouraged to read the User Manual before using the BAT. If you “crash the BAT”, e.g., run-time error or over-flow error or other VBA-based error messages are displayed, please close the file (the results may not be reliable) and report this issue through the BAT response form at www.arnotresearch.com.

The BAT is implemented (coded) in Visual Basic for Applications (VBA) and the Graphical User Interface is designed in Excel™. The BAT allows the user to input various **Lines of Evidence (LoE)** to support the overall bioaccumulation assessment. Most LoE require the completion of Data Evaluation Templates (DETs) to determine **reliability** scores. For a full bioaccumulation assessment using the BAT, the user will follow the five steps detailed below. These steps are described in more detail throughout this document.

1. Opening the BAT, Initialization, assigning **Relevance Weighting** and **Classification Threshold** values
2. Addition of physical-chemical property information (initial user entry form and summary sheet)
3. Addition of information on biotransformation and/or dietary absorption efficiency (E_D , fraction of chemical absorbed across the gut); the user can opt to assume total persistence (enter no data) or at minimum enter a QSAR estimate of the biotransformation half-life for fish and/or mammals.
4. Definition of scaling factors for determining biotransformation rates in autotrophs and invertebrates relative to those in fish
5. Addition of other LoE for B-metrics (e.g., BCF-QSAR, Laboratory BCF study, Laboratory BMF study, Rat-TK study, field studies, etc.)
6. Generation of **Final Summary** Results

Step 1: Initialization, Relevance Weighting and Threshold Values for Categorization

For the first time opening the BAT after downloading or if the file you've opened displays:

 SECURITY RISK Microsoft has blocked macros from running because the source of this file is untrusted. [Learn More](#)

We have developed the BAT code only for carrying out the model calculations and functionality within the workbook and it can be trusted. To unblock the code and make the BAT useable, start at step 1, below - otherwise you may start at step 2.

1. Right click on the BATver2.1.xlsb file and select **Properties**. Check the **Unblock** checkbox. Click **OK**.
2. Open the BATver.2.1.xlsb file.
3. The Start Page will open. Depending on your security settings it may be necessary to **Enable Content** for the VBA code and BAT to work.

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4. After pressing “Enable Content” it may take a few seconds for the BAT to load. Please be patient and do not touch any buttons until the initialization form loads.
 5. A dialogue box will open providing two options, i) Open Quick Start PDF and ii) Open BAT.
 6. After clicking on the “Open BAT” button, the “BAT Initialization” Form will appear. Enter the following information (information can be “copied” and “pasted” in):
 - Chemical Name
 - CAS Number (enter “NA” if not available)
 - SMILES (this can be left blank but is recommended to increase transparency for the chemical structure that is being evaluated in the BAT).
 7. Select “Neutral” if the chemical is a neutral organic, or “Ionic” if the chemical is an ionizable organic chemical (IOC).
 8. Identify yourself in the Assessor’s Name and Organization textboxes (for display in the Report).
 9. Regulation scenario:
 - **Option 1:** Select the appropriate regulatory scenario from the drop-down box; this will auto-populate the threshold values in the right column. It is recommended that the user confirm these values are accurate and representative of the regulatory scenario selected.
 - **Option 2:** Separately select or enter the applicable threshold values in the right-hand column, as desired. You may enter a name/acronym for your own regulatory scenario in the drop-down box (will not be saved for future assessments).
 10. Assign **Relevance Weighting** to the various B metrics:
 - User enters relevance weighting based on their discretion (judgement); values of 5 indicate high relevance, values of 0 indicate low relevance.
 - See **User Manual** page 11 for guidance on this step.
 11. Click the “Initialize BAT” button.
 12. A box will open to allow you to save your work to the file directory in which the BAT file exists - press “Save” for the default file name (or change the name and press “Save”). You may change the file name (and location on the computer) but **it is mandatory to save the file as an Excel Binary Workbook, *.xlsb** (default).

The BAT can be saved and the file closed and re-opened after this initiation stage.
 13. A new worksheet (tab) will open entitled **BAT Main** - this worksheet is the main interface for navigating the tool and entering all data used by the BAT (physical-chemical properties, biotransformation data, bioaccumulation data).
 14. From this point forward you may return to the **Start** sheet to view the links to this Quickstart Guide, the Manual, the Software License and the list of Updates. Click the corresponding button to access these documents.

Step 2: Physical-Chemical Properties (Neutral Chemical)

1. Click on the “Physical-Chemical Properties” button to open the initial user-entry form for data entry. Enter physical-chemical property information (measured or modelled estimates).

The user can access Physical Chemical property data from EAS-E Suite at www.eas-e-suite.com and other databases

- **MINIMUM** requirements are:
 - Molecular weight (g/mol)
 - Water solubility (mg/L)
 - Log K_{OW} (required for spLFER, optional for ppLFER)
- AND
- Henry’s Law constant OR
 - Log K_{AW} OR
 - Log K_{OA}
2. Select an approach for estimating partitioning to biological phases (if applicable) (see **User Manual** pages 15-16 for more information):
 - spLFER
 - ppLFER
 3. If desired, enter data for the properties listed in the Optional Inputs section of the user form. The user may update the Enthalpies of Phase Change values from the defaults presented. These are utilized in temperature-correction of partitioning throughout the BAT given environmental conditions for each study.
 4. Click the “Enter Data into BAT....” button.
 - Minimal required inputs still missing will be identified by yellow highlighting; enter the appropriate data and press the “Enter Data into BAT....” button again. You will be taken to the **Chemical Summary** sheet, where data on properties are summarized and displayed.
 5. Press the “Return to BAT Main...” button to enter biotransformation, dietary absorption efficiency (E_D) and bioaccumulation data. **The user cannot enter information on biotransformation in the chemical property sheet; this will be done by the BAT following the next stage of user input.**
 6. The **BAT Main** sheet will open; you can now enter data on Biotransformation (Step 3: Empirical In Vivo, In Silico QSAR, In Vitro S9, In Vitro HEP, In Vitro Microsomal and In Vivo E_D), set the assumptions for autotroph and invertebrate biotransformation (Step 4) or click on the “Next” button to calculate other B-metrics and enter other bioaccumulation data (Step 5).

Note: Clicking on the “Next” button also triggers the BAT to conduct the in silico bioaccumulation assessment using the built-in modelling approaches described in the User Manual. The calculations are driven by the physical-chemical properties and any biotransformation data entered by the user (see below). If biotransformation studies are entered AFTER the “Next” button is clicked, the user is prompted to update existing bioaccumulation estimates to update the built-in BAT model calculations. In the absence of any user-entered biotransformation data, the chemical is assumed to be persistent. Accordingly, the BAT-calculated bioaccumulation metrics are expected to be conservative for chemicals that are subject to biotransformation in reality.

Step 2: Physical-Chemical Properties (Ionizable Chemical)

Click on the “Physical-Chemical Properties” button to open the Initial user-entry form for data entry. Enter physical-chemical property information (measured or modelled estimates).

The user can access Physical Chemical property data from EAS-E Suite at www.eas-e-suite.com and other databases

- **MINIMUM** requirements are:
 - pKa and whether the chemical is an acid or a base
AND
 - Molecular weight (g/mol)
 - Intrinsic Water solubility **of neutral form** (mg/L)
AND
 - Henry’s Law constant OR
 - Log K_{AW} OR
 - Log K_{OA} **of neutral form**
- The user can choose to enter log $K_{OW,N}$ of neutral form or open the “Neutral + Charged Form” tab on the user-entry form and enter the octanol-water distribution ratio, D_{OW} (required for spLFFER, optional for ppLFFER).
- The user can also enter other distribution ratios on the same tab of the user-entry form (see **User Manual** pages 17 - 20 for more information).

Follow steps 2 - 6 as for neutral chemicals above.

Step 3: Biotransformation

1. The user may **assume** that the chemical has total persistence in biota by clicking on the “Next” button (which activates the buttons in the Bioaccumulation Data area of the **BAT Main** sheet). However, it is recommended that users evaluate the chemical with some consideration for biotransformation (e.g., at least using in silico predictions).
2. Click on the button corresponding to the first type of biotransformation you have available. All empirical dietary absorption efficiency (E_D), In Vivo and In Silico (QSAR) biotransformation half-life data or data are entered on a single corresponding sheet (Step 3A, 3B and 3C respectively). Please note that if you have multiple in vitro biotransformation studies / inputs, you will input them individually on different **In Vitro** sheets (Step 3D-3F).

The user can access In Vivo, In Vitro and In Silico biotransformation estimates from EAS-E Suite at www.eas-e-suite.com and other databases

The user may return to update or enter additional biotransformation information even AFTER bioaccumulation metrics have been estimated/entered.

The BAT “Next” button must be subsequently re-clicked to update the BAT estimates.

Return to the **BAT Main** to input other types of biotransformation information. The various options are provided in Steps 3A-3F.

Step 3A: Empirical In Vivo Dietary Absorption Efficiency Data

1. Enter the organism type (type in fish, herbivore or carnivore).
2. Enter the relevant information from the in vivo study.
 - Source
 - Species
 - Dietary absorption efficiency, E_D (%)
3. Evaluate the reliability of the study by completing the DET; the DET is presented as a series of questions for the users to answer manually, visible by scrolling to the right side of the worksheet.
4. Repeat as necessary to enter all available data and complete the associated DETs.
5. Click on the “Assess Reliability...” button to tabulate scores for all entered studies (displayed in column I). You may add new studies and change existing studies or DETs; you must click the “Assess Reliability...” button to update the worksheet.
6. Return to the **Chemical Summary** sheet by clicking on the “I’m done! Add all data to BAT” button. User-entered biotransformation half-life data are converted to mass- and temperature- standardized rate constants and displayed in the Biotransformation summary area of the sheet.
7. Click on the “Return to **BAT Main...**” button to enter other types of data.

Step 3B: Empirical In Vivo Biotransformation Data

The user can access In Vivo biotransformation estimates from EAS-E Suite at www.eas-e-suite.com and other databases

1. Enter the organism type (type in fish or mammal).
2. Enter the relevant information from the in vivo study.
 - Source
 - Species
 - Biotransformation half-life (days)
 - Mass of organism (kg)
 - Temperature (°C)
3. Evaluate the reliability of the study by completing the DET; the DET is presented as a series of questions for the users to answer manually, visible by scrolling to the right side of the worksheet.
4. Repeat as necessary to enter all available data and complete the associated DETs.
5. Click on the “Assess Reliability...” button to tabulate scores for all entered studies (displayed in column I). You may add new studies and change existing studies or DETs; you must click the “Assess Reliability...” button to update the worksheet.
6. Return to the **Chemical Summary** sheet by clicking on the “I’m done! Add all data to BAT” button. User-entered biotransformation half-life data are converted to mass- and temperature- standardized rate constants and displayed in the Biotransformation summary area of the sheet.
7. Click on the “Return to **BAT Main...**” button to enter other types of data.

Step 3C: In Silico Biotransformation QSARs

The user can access In Silico biotransformation estimates from EAS-E Suite at www.eas-e-suite.com and other databases

1. Enter the organism type (user should type in fish or mammal).
2. Enter the relevant information from the QSAR model.
 - Source
 - QSAR Description (to identify the specific QSAR used)
 - Biotransformation Half-life (days)
 - Mass of organism (kg)
 - Temperature (°C)
3. Evaluate the reliability of the QSAR by completing the DET; the DET is presented as a series of questions for the users to answer manually, visible by scrolling to the right side of the worksheet.
4. Repeat as necessary to enter all available data and complete the associated DETs.
5. Click on the “Assess Reliability...” button to tabulate scores for all entered studies (displayed in column I). You may add new studies and change existing studies or DETs; you must click the “Assess Reliability...” button again to update the worksheet.
6. Return to the **Chemical Summary** sheet by clicking on the “I’m done! Add all data to BAT” button. NOTE: User-entered biotransformation half-life data are converted to mass- and temperature- standardized rate constants and displayed in the Biotransformation summary area of the sheet.
7. Click on the “Return to **BAT Main...**” button to enter other types of data.

Step 3D: In Vitro S9 Biotransformation Study

The user can access In Vitro S9 biotransformation estimates from EAS-E Suite at www.eas-e-suite.com and other databases

1. Select an organism type (fish, rodent or human).
2. Input study information.
 - MINIMUM requirements are:
 - Mass of organism (g)
 - Liver S9 protein content (default value provided)
 - Protein concentration
 - Reaction temperature
 - At least one of:
 - Slope (log₁₀ concentration vs time in h) OR
 - First-order rate constant (k_e , 1/h) OR
 - Intrinsic In vitro clearance rate ($CL_{InVitro,INT}$, mL/h/mg protein)
 - Additional information on the study can be input if the information is available.

-
3. The assay fractional composition is estimated based on the reported protein concentration information and the lipid and lipid:protein ratios shown on the sheet. The calculated assay fractional composition can be overwritten by the user.
 4. You can input or select a default assumption ("Nichols et al. 2013" or "Composition") for fraction unbound in the test system using the drop-down menu. See **User Manual** pages 27-31 and **Appendix A3** for additional information.
 5. To calculate rate constants, clearances and half-lives and evaluate the reliability of the in vitro study, click on the "Assess Study Reliability HERE" button"; the DET is presented as a user-entry form ("*Data Quality Criteria for Data Reliability of an S9 in vitro biotransformation assay*") that will open with a series of questions to be answered. Scroll down to finish all questions, then click on the "Assess Study Reliability" button at the bottom of the user form; the reliability score will be reported in the Assessment area of the worksheet.
 6. Click on the "Then Calculate IVIVE" button to proceed to the next stage.
 - Select IVIVE parameters
 - Default parameters ("Default organism") from Nichols et al., 2013 [this selection will auto-populate the values in column D] OR
 - You can enter preferred (study specific) values to override the defaults
 - Select a method to calculate blood-water partitioning, P_{BIW} [these selections will auto-populate the values in column D]
 - Equilibrium partitioning OR
 - Fitzsimmons et al., (2001) (**not recommended for IOCs**). select or enter appropriate values for fraction unbound in whole blood and ratio of fractions unbound in whole blood vs. test system.
 - Calculated values for in vivo intrinsic clearance, hepatic clearance and whole-body biotransformation rate constant will appear.
 7. Click on the "Return to in vitro DET" button to return to the in vitro S9 entry sheet.
 8. Click on the "I'm done with this study" button to return to the **Chemical Summary** sheet; the whole-body biotransformation rate constant (standardized to size and temperature) generated by the BAT will be displayed in the Biotransformation summary area.
 9. Click on the "Return to **BAT Main**..." button to enter additional studies and continue the analysis.

Step 3E: In Vitro HEP Biotransformation Study

The user can access In Vitro HEP biotransformation estimates from EAS-E Suite at www.eas-e-suite.com and other databases

1. Select an organism type (fish, rodent or human).
2. Input study information.
 - MINIMUM requirements are:
 - Mass of organism (g)
 - Liver hepatocyte content (default value provided)
 - Cell concentration (10^6 cells/ml)
 - Reaction temperature

- At least one of:
 - Slope (log10 concentration vs time in h) OR
 - First-order rate constant (k_e , 1/h) OR
 - Intrinsic In vitro clearance rate ($CL_{InVitro,INT}$, mL/h/10⁶ cells)

Follow the steps outlined above for in vitro S9 studies to complete data entry, assess the study, return to the **Chemical Summary** sheet and return to the **BAT Main** sheet.

Step 3F: In Vitro Microsomal Biotransformation Study

The user can access In Vitro HEP biotransformation estimates from EAS-E Suite at www.eas-e-suite.com and other databases

1. Select an organism type (fish, rodent or human).
2. Input study information.
 - MINIMUM requirements are:
 - Mass of organism (g)
 - Liver microsomal protein content (default value provided)
 - Protein concentration
 - Reaction temperature
 - At least one of:
 - Slope (log10 concentration vs time in h) OR
 - First-order rate constant (k_e , 1/h)
 - Intrinsic In vitro clearance rate ($CL_{InVitro,INT}$, mL/h/mg protein)

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Follow the steps outlined above for in vitro S9 studies to complete data entry, assess the study, return to the **Chemical Summary** sheet and return to the **BAT Main** sheet.

Step 4: Enter Invertebrate/Autotroph Biotransformation Scaling Factors

1. Once all fish and mammal biotransformation information are entered, on the **BAT Main** sheet click the “4. Define Invert/Autotroph Biotrans HLs” button
2. The BAT will automatically open the **InvertAutotroph-BioTrans 1** sheet.
3. Enter a scaling factor in the first box to scale the average fish biotransformation rate for use for invertebrates and another in the second box for autotrophs:
 - Default for invertebrates = 3 (i.e., fish HL_B multiplied by three, then scaled to organism size for invertebrates)
 - Equal to fish biotransformation rate (although scaled to organism size for invertebrates) = 1
 - Default for autotrophs is the assumption of total persistence (no biotransformation) = 0
 - Other

Step 5: Enter Bioaccumulation Lines of Evidence

1. Return to the **BAT Main** sheet and use the checkboxes to select whether or not to output BAT in silico BMFs for homeotherms and whether to output growth-corrected Lab BMFs and Lab BCFs.
2. Click on the “Next” button.
 - The BAT will automatically complete the BAT in silico bioaccumulation assessment for 9 aquatic and 3 terrestrial-based generic B-metrics (see **User Manual** pages 52-57 and **Appendix A7**) using the provided property and biotransformation data.
3. Select the type of Bioaccumulation Line of Evidence to enter (Laboratory BCF, Laboratory BMF, Field BAF/BMF, Field TMF, BCF QSARs) and follow the instructions below.

Step 5A: Laboratory Fish BCF Study

1. Input study information.
 - Provide study reference information (author, year, name of study).
 - **MINIMUM** additional requirements for a **steady-state lab BCF** are:
 - Mass (end) (g)
 - Fish lipid content (%)
 - Test concentration C_w (mg/L)
 - Select “freely dissolved” or “total” water concentration definition from pull-down menu
 - **MINIMUM** additional requirements for a **kinetic lab BCF** are:
 - Uptake period (days)
 - Depuration (days)
 - Mass (start) (g)
 - Mass (end) (g)
 - Fish lipid content (%)
 - Total elimination rate constant, k_T (1/d)
 - Test concentration C_w (mg/L)
 - Select “freely dissolved” or “total” water concentration definition from pull-down menu
 - Additional information on the study can be input if the information is available. One or both study type BCFs can be entered, however only one can be selected to display in the results from each study entry. For more than one BCF measured/calculated per study, enter a separate Laboratory BCF Study for each value to be considered for the assessment.
2. To calculate various rate constants and BCF metrics and evaluate the reliability of the laboratory BCF study, click on the “Assess Input and Reliability” button; the DET is presented as a user-entry form (“*Quality Criteria for Data Reliability of a BCF study*”) that will open with a series of questions to be answered. Scroll down to finish all questions, then click on the “Assess Study Reliability” button at the bottom of the user form. Depending on data entered (kinetic BCF vs steady-state BCF, LC50), a series of notifications and questions may appear in dialogue boxes for you to consider; once finished with these dialogue boxes, the reliability score will be reported in the Assessment area of the sheet. See **Note**, below.
3. Click on the “Add Data to BAT” button to select a single BCF value to represent this study on the **Summary Results** table and return to the **BAT Main** sheet.

Note: Various rate constants and BCF metrics automatically calculated by the BAT based on physical-chemical properties and biotransformation rate constants are also displayed on the Laboratory BCF sheet in the area below the reliability score if enough data are entered to calculate them.

Step 5B: Laboratory Invertebrate BCF Study

4. Input study information.

- Provide study reference information (author, year, name of study).
- Species identification
- **MINIMUM** additional requirements for a **steady-state lab invertebrate BCF** are:
 - Average mass per sample (g)
 - Lipid content (%)
 - Test concentration C_w (mg/L)
 - Select “freely dissolved” or “total” water concentration definition from pull-down menu
- **MINIMUM** additional requirements for a **kinetic lab BCF** are:
 - Uptake period (days)
 - Depuration (days)
 - Average mass per sample (g)
 - Lipid content (%)
 - Total elimination rate constant, k_T (1/d)
 - Test concentration C_w (mg/L)
 - Select “freely dissolved” or “total” water concentration definition from pull-down menu
- Additional information on the study can be input if the information is available. One or both study type BCFs can be entered, however only one can be selected to display in the results from each study entry. For more than one BCF measured/calculated per study, enter a separate Laboratory BCF Study for each value to be considered for the assessment.

5. To calculate various rate constants and BCF metrics and evaluate the reliability of the laboratory BCF study, click on the “Assess Input and Reliability” button; the DET is presented as a user-entry form (“*Quality Criteria for Data Reliability of an Invertebrate BCF study*”) that will open with a series of questions to be answered. Scroll down to finish all questions, then click on the “Assess Study Reliability” button at the bottom of the user form. Depending on data entered (kinetic BCF vs steady-state BCF, LC50), a series of notifications and questions may appear in dialogue boxes for you to consider; once finished with these dialogue boxes, the reliability score will be reported in the Assessment area of the sheet.

6. Click on the “Add Data to BAT” button to select a single BCF value to represent this study on the **Summary Results** table and return to the **BAT Main** sheet.

Step 5C: Laboratory BMF study (Fish)

1. Input study information.

- Provide study reference information (author, year, name of study)
- Species name
- **MINIMUM** a BMF_{SS} , $BMF_{SS,5\%}$, BMF_K , $BMF_{K,G}$, $BMF_{K,5\%}$ or $BMF_{K,5\%,G}$

- **MINIMUM** additional requirements to calculate a **steady-state lab BMF** are:
 - Mass (end) (g)
 - Fish lipid content (%)
 - Test concentration in diet (mg/kg)
 - Concentration in fish at end of uptake phase (mg/kg)
 - **MINIMUM** additional requirements to calculate a **kinetic lab BMF** are:
 - Uptake period (days)
 - Depuration (days)
 - Mass (start) (g)
 - Mass (end) (g)
 - Fish lipid content (%)
 - Total elimination rate constant, k_T (1/d)
 - Test concentration in diet (mg/kg)
 - Concentration in fish at end of uptake phase (mg/kg)
 - Additional information on the study can be input if the information is available. One or both study type BMFs can be entered, however only one can be selected to display in the results from each study entry. For more than one BMF measured/calculated per study, enter a separate Laboratory BMF Study for each value to be considered for the assessment.
2. To calculate various rate constants and BMF metrics and evaluate the reliability of the laboratory BMF study, click on the “Assess Input and Reliability” button; the DET is presented as a user-entry form (“*Quality Criteria for Data Reliability of a BMF Study*”) that will open with a series of questions for the user to answer. Scroll down to finish all questions, then click on the “Assess Study Reliability” button at the bottom of the user form. Depending on data entered (kinetic BMF vs steady-state BMF, etc.), a series of notifications and questions may appear in dialogue boxes for you to consider; once finished with these dialogue boxes, the reliability score will be reported in the Assessment area of the sheet. See **Note** below.
 3. Click on the “Add Data to BAT” button to select a single BMF value to represent this study on the **Summary Results** table and return to the **BAT Main** sheet.

Note: Various rate constants and BMF metrics automatically calculated by the BAT based on physical-chemical properties and biotransformation rate constants are also displayed on the Laboratory BMF sheet in the area below the reliability score if enough data are entered to calculate them.

Step 5D: Rodent Toxicokinetic (TK) Study for Mammalian BMF Estimation

1. Input study information.
 - Provide study reference information (author, year, name of study)
 - Species name
 - **MINIMUM** requirements to calculate a **kinetic lab BMF** from TK data are:
 - Total elimination rate constant, k_T (1/d) OR
 - Total elimination half-life HL_T (d)
 - Default values for organism lipid content, feed composition, feeding rate and absorption efficiencies are used unless entered by the user. Users are encouraged to enter study specific values rather

than use the defaults if possible. Additional information on the study can be input if the information is available.

2. To calculate BMF metrics and evaluate the reliability of the laboratory BMF study, click on the “Assess Input and Reliability” button; the DET is presented as a user-entry form (“*Quality Criteria for Data Reliability of a Rodent TK Study*”) that will open with a series of questions for the user to answer. Scroll down to finish all questions, then click on the “Assess Study Reliability” button at the bottom of the user form. A series of notifications and questions may appear in dialogue boxes for you to consider; once finished with these dialogue boxes, the reliability score will be reported in the Assessment area of the sheet.
3. Click on the “Add Data to BAT” button to select a single BMF value to represent this study in the **Summary Results** table and return to the **BAT Main** sheet.

Step 5E: Field BAF/BMF study

1. Input study information.

- Provide study reference information (author, year, name of study)
- **MINIMUM** input requirements to enter for a **field BAF study** are:
 - Organism (species) name AND
 - Organism type: invertebrate, fish, homeotherm-herbivore, or homeotherm-omni/carnivore AND
 - BAF OR
 - BAF_L (BAF_L will be calculated from BAF, if lipid content is included as input) OR
 - BAF_{fdL} (BAF_{fdL} will be calculated from BAF, if lipid content and organic carbon concentrations are included as input)
- If you want the BAT to calculate BAFs from field data, the following information is required:
 - Concentration of chemical in biota ($\mu\text{g}/\text{kg}$)
 - Concentration of chemical in water (total, $\mu\text{g}/\text{L}$) OR
 - Concentration of chemical in water (freely dissolved, $\mu\text{g}/\text{L}$)
 - Organism lipid content (%) (required for BAF_L and BAF_{fdL})
 - Information on organic carbon concentrations in water (required for BAF_{fdL} if dissolved water concentrations are not already entered)
- Additional (optional) study information for BAFs
 - Organism mass
 - Water pH
 - Water temperature
- **MINIMUM** input requirements for a **field BMF study** are:
 - Organism (species) name (enter in Rows 17 to 24) AND
 - Organism type: invertebrate, fish, homeotherm-herbivore, or homeotherm-omni/carnivore AND
 - BMF OR
 - BMF_L (BMF_L will be calculated from BMF if lipid content is included as input)
- If you want the BAT to calculate BMFs from field data, the following information is required:
 - Concentration of chemical in biota ($\mu\text{g}/\text{kg}$) (enter in Rows 17 to 24 as necessary)
 - Consumer (Predator) lipid content (%) (required for BMF_L ; enter in Rows 17 to 24 as necessary)

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- Concentration of chemical in diet/prey ($\mu\text{g}/\text{kg}$) (enter in Rows 28 to 35 as necessary)
 - Diet (Prey) lipid content (%) (required for BMF_L; enter in Rows 28 to 35 as necessary)
 - Feeding preferences/matrix (see **User Manual** page 46)
 - Additional (optional) study information for BMFs
 - Organism mass
 - Water pH
 - Water temperature
 - Concentration of chemical in water (total, $\mu\text{g}/\text{L}$)
 - Information on organic carbon concentrations in water
2. Click on the “Assess Input and Reliability” button to calculate field BAFs and BMFs from user entered data and access the *Quality Criteria for Data Reliability of a BMF/BAF Field Study* form.
 3. Click the “Add Data to BAT” button to select a single value for each organism to be added to the **Summary Results** table and return to the **BAT Main** sheet.

Step 5F: Field TMF Study

1. Input study information.
 - Provide study reference information (Author, year, name of study).
 - Enter the type of food web/top predator (invertebrate, fish, homeotherm-herbivore or homeotherm-omni/carnivore)
 - Enter the reported TMF from the study of interest in the box indicated OR
 - MINIMUM requirements for BAT to calculate a TMF from field data are:
 - Organism (species) name AND
 - Lipid-normalized concentration of chemical in biota ($\mu\text{g}/\text{kg}$) AND
 - Trophic position
 - An option is to enter the wet weight concentrations and the lipid contents for the food web organisms and BAT will calculate the lipid-normalized concentrations by clicking on the *Calculate lipid-normalized concentrations* button.
 - Then click on the “Calculate TMF” button
2. Click on the “Assess Input and Reliability” button to access the *Quality Criteria for Data Reliability of a TMF Field Study* form; the reliability score of the study is also tabulated.
3. Click the “Add Data to BAT” button to add the TMF to the **Summary Results** table return to the **BAT Main** sheet.

Step 5G: BCF QSARs (Aquatic Organisms)

1. Enter the relevant information from the QSAR model.
 - Source
 - Name of QSAR
 - BCF (L/kg)
 - Information on related publication in peer-reviewed or grey literature, if available (Author, Year, Title, Source)

2. Evaluate the reliability of the BCF QSAR by completing the DET; the DET is presented as a series of questions for you to answer manually (entering Y/N), visible by scrolling to the right side of the worksheet.
3. Repeat as necessary to enter all available data and complete the associated DETs.
4. Click on the “Assess Reliability” button and the resulting reliability scores for all entries will be shown in Column H. You may add new studies or change existing ones. Click the “Assess Reliability” button again to update the results in Column H.
5. Click on the “Add Data to BAT” button to add the entered BCFs to the **Summary Results** table and return to the **BAT Main** sheet.

Step 6. View Final Summary Results

1. **Results worksheet (tabular):** A full explanation of the Results Summary worksheet is provided in the **BAT User Manual**. In brief, this sheet provides:
 - A summary table with the LoE entered by the user and/or calculated by the BAT including:
 - A description of the bioaccumulation metric (LoE) within the BAT.
 - Individual values for each type of LoE entered or calculated for each study (kinetic; kinetic, lipid std/norm; kinetic, growth corrected; kinetic, lipid std/norm, growth corrected; steady-state; steady-state, lipid std/norm)
 - A corresponding list of the data quality considerations that were not met/fulfilled for a given parameter. The numbers listed correspond to the question # on the DETs.
 - The numerical value of the bioaccumulation metric selected. Upper and lower range estimates of this value are also provided for built-in BAT model calculated (in silico) bioaccumulation metrics based on variance in biotransformation half-lives and dietary absorption efficiency (E_D) entered by the user, if multiple values for these parameters exist..
 - The “**B**” **Category** (nB, B, vB) for each LoE based on the user-defined **Categorization Thresholds** determined *a priori* in Step 1.
 - The **Relevance Weighting** assigned to the bioaccumulation metric (LoE) based on the user-defined relevance weighting scores in Step 1 (values between 0 - 5).
 - The **Reliability Score** for the LoE as determined from the data quality considerations outlined in the corresponding DETs (scaled to values between 0 - 5).
 - The **Confidence Factor** of the selected bioaccumulation metric value due to input (biotransformation and E_D) variability.
 - **Chemical activity ratios** (if applicable, e.g., not applicable to IOCs) for each LoE
 - The overall **Strength of Evidence** (expressed as %) for a particular “B” classification. There are three **Strength of Evidence** summaries:
 1. All LoE generated (i.e., BAT-calculated) and user-entered (e.g., lab BCFs, field BAFs, etc)
 2. Only user-entered LoE; no BAT-calculated LoE
 3. All LoE, *except* terrestrial B metrics
2. **Change Relevance and/or Thresholds** button brings up the BAT Initialization form, pre-filled with user selected values. The user may change relevance weighting values and/or nB/B/vB threshold values. When the **Update Values** button is clicked, any changes are updated in the **Summary Results** table and

in the Report, including nB/B/vB determinations and **Strength of Evidence** table if thresholds are changed.

3. Graphical output: Click on the “View Graphical Results” button to view.

- A plot (red-yellow-green-blue) showing the values of key LoE against user-defined thresholds on the Y-axis with the reliability scores (scaled to values between 0 - 5) on the x-axis; BAT in silico output is presented in the blue area of the plot (no reliability score assigned). The organism type of each LoE is indicated: invertebrate, fish, herbivore (homeotherm), and omni/carnivore (homeotherm).
- Benchmarking plots: assessed chemical against aquatic ‘B’ metrics for PCBs and HCB. Click on the “View Graphical Results” button to view:
 - Log BAFs vs. log K_{OW}
 - Log lab BCF vs. log K_{OW}
 - Lab BMF (fish) vs. log K_{OW}

4. PDF Report: The PDF Report collects and summarizes all key inputs and BAT outputs in a format suitable for printing. Data include information from the **Initialization** sheet (B thresholds and relevance weightings), physical-chemical property sheet, biotransformation data and bioaccumulation data as well as the figures displayed in the **Graphical Results** sheet.

5. HL_T Sheet: The Total Elimination Half-life for each organism assessed in each B-metric is calculated and output based on the elimination data given in each study.

Updates in BAT Ver.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 HLA_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 Ver.2.0

Updates to BAT input:

- Option to enter chemical-specific enthalpies of phase change to improve temperature correction of partitioning behaviour
- Increased capacity to enter 25 studies per LoE
- Addition of an unlocked “Work Area” on various study input sheets; addition of “Work Area” unlocked sheet to copy/paste/enter additional data as needed for the assessment
- Greater flexibility to add/adjust input and biotransformation information after the BAT Estimated and study LoE have been entered; these results must be run again to update any output affected by changes in the input; pop up facilitating this is included.

- ☑ Chemical degradation in the gastro-intestinal tract (GIT) estimated from entry of Dietary Absorption Efficiency (E_D) study entry sheet
- ☑ Invertebrate BCF LoE study entry sheet and DET form
- ☑ Rodent TK BMF LoE study entry sheet and DET form
- ☑ Organism type and/or species identification added as required input to study LoE
- ☑ If the study LoE is known, it can be entered without the data necessary to calculate it
- ☑ In Vitro and In Vivo data reliability forms and scoring have been updated/included to be consistent with associated databases included in EASE-Suite
- ☑ *In Vitro* assay composition automatically calculated based on reported protein concentrations; these can be overwritten by the user as well.
- ☑ Fugacity ratio calculations are based on total sorption capacity of organism and diet (where applicable)
- ☑ Refinement to input sheet indications for “required for calculation” inputs on all forms
- ☑ Updates to Field data scoring and method detection limit (MDL) considerations
- ☑ Added button to clear all distribution ratios on ionic physical-chemical input form
- ☑ Added button to optionally calculate required lipid-normalized concentrations on **Field TMF** input sheet

Updates to BAT output:

- ☑ To avoid pseudo-replication influencing the **Weight of Evidence** (WoE), a single estimate is selected for each BAT estimate and there is a “pop-up” to allow the user to select a single representative value for each entered LoE; all entered/generated values are shown in the **Summary Results** table, but only the selected values are compared to threshold values and assessed.
- ☑ Integrate output for generic lab invertebrate BCF, BMF and lab rat BMF into **Summary Results**.
- ☑ Integrate output for generic field aquatic mammal (Seal) BMF into **Summary Results**
- ☑ All mammals and birds are deemed “homeotherms-herbivore” or “homeotherms-omni/carnivore” based on their diet
- ☑ Update 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”

Updates to BAT calculations:

- ☑ Internal foodweb updates to include:
 - Dietary absorption efficiency calculation update to Arnot and Mackay 2018
 - Bioenergetic and water balances aquatic and benthic invertebrates in aquatic foodweb aquatic mammal (seal) included in aquatic foodweb
- ☑ Internal foodweb includes separate laboratory conditions for: aquatic invertebrates, fish, and rat
- ☑ Fugacity Ratio calculations updated and determined separately for steady-state or kinetic LoE
- ☑ Biotransformation HL for each organism type (fish and mammals) is calculated as the weighted geometric mean of the biotransformation HLs reported in the BAT. The weighting is based on both study type (in Vitro, In Vivo, In Silico) and study-specific reliability scores.
- ☑ E_D for each organism type (fish and herbivores and omni-/carnivores) is calculated as weighted geometric mean of the study the biotransformation HLs reported in the BAT. The weighting is based on study-specific reliability scores.
- ☑ Temperature corrections to environmental and biological partition coefficients for neutral and ionic chemicals occur throughout the BAT based on the generic (BAT-Estimated) and reported (study) organism and environmental temperatures.
- ☑ added the compositional approach as an option for estimating f_u for *In Vitro* studies
- ☑ Correction: Zdiet for mammals consuming fish were consuming ONLY the neutral form in previous versions. This is corrected in Ver.2.0

Updates in BAT Ver.2.01

- ☑ Counter of user entered studies for strength of evidence table now only counts if $RS > 0$
- ☑ Organism temperature used for fish bioenergetics equations to differentiate lab and field conditions
- ☑ Addition of User-defined weighting of biotransformation study types to Chemical Summary Sheet
- ☑ Addition of option for user defined or scaling-factor-from-fish biotransformation for invertebrate and plant/autotroph biotransformation rates. Default remains as "persistent"
- ☑ Extra "theta" removed from BAF_{SS} calculation (input was updated to use dissolved fraction for concentration in Jan2021, so "theta" is redundant)
- ☑ Steady State and Kinetic for lab B-metrics calculations aligned

Updates in BAT Ver.2.02

- ☑ Update modelled lab fish growth and feeding preferences to reflect existing databases (Arnot and Quinn 2015, Arnot and Gobas 2006)
- ☑ Plant and Invertebrate biotransformation scaling factor adjustments
- ☑ Option for output of growth corrected lab results
- ☑ Updates to k_1 and k_D calculations for homeotherms

Updates in BAT Ver.2.1

Physical-chemical input expansion

- Any/all biotic partition coefficients/distribution ratios can now be parameterized by the user on the physical-chemical input form.
- References to "bovine serum albumin" (e.g. K_{BSA}) were replaced with "serum albumin" (e.g. K_{SAW}).
- All spLFER partition coefficient calculations were aligned to reflect the temperature at which they were developed and were then adjusted to the various temperatures in different environments in the BAT.
- Input and output of ΔHOC (enthalpy of phase change for organic carbon) was added.
- Either spLFERs or ppLFERs can be used for biopartitioning; the proportionality constant approach for non-lipid organic matter (NLOM) is no longer used.
- Specific fractions of organic carbon and detritus in organism and diet items were revised.
- Dietary assimilation efficiencies for dietary absorption of lipids, proteins, carbohydrates and water were refined.
- Added new energy densities explicitly for organic carbon in phytoplankton (18 kJ/g C) and detritus (20 kJ/g C).

Chemical uptake efficiency calculations

- Aquatic and benthic invertebrate E_D and chemical absorption efficiency from water (E_W) are now calculated using the same model used for fish.
- Laboratory DET E_W calculations updated to use the same as above.
- Lab rat E_D is calculated as an omnivore/carnivore (previously assumed herbivore).

Specific consideration of particulate organic matter (POM) and dissolved organic matter (DOM) in water

- POM and DOM added as specific subfractions of natural and laboratory waters.
- Mass fraction of OC is 0.2 in POM and 0.5 in DOM.
- POM concentration in laboratory water now 0 mg/L, and DOM concentration changed to 2 mg/L.

Updates and additions to specific organisms/compartments

- Generic lab fish growth, respiration and feeding rates are calculated using bioenergetic equations based on the lab feeding rate, a growth rate uncertainty factor of “/10”, and a metabolic activity factor of 1.
- Added new laboratory-specific temperatures for water (at 15 °C) and air (at 23 °C) to parameterize relevant lab fish, invertebrate and rat Zvalues and fugacities.
- Updated seal parameterization to align with the model in the Bioaccumulation Estimation Tool (BET) in the Exposure And Safety Estimation (EAS-E) Suite platform (www.eas-e-suite.com).
- Polar bear that consumes a seal was added to the BAT food web model; however, these calculations are not presented in the BAT modelling results.

Alignment of calculations with the BET in EAS-E Suite.

- Change in calculation of aerosol-air partitioning using temperature-corrected K_{OA} .
- Change in calculation of K_p and K_d for aerosol-air and solid-water partitioning.
- Change in calculation of K_{orgW} for biotic subfractions.
- Parameterization of volume fractions for generic freshwater biota in the bulk water compartment.
- Renamed fugacity ratios to chemical activity ratios.

Updates to In Vitro and IVIVE study input sheets and calculations

- Removal of “ $f_U = 1$ ” option on all drop-down menus.
- Renamed “BAT-calculated” as “Nichols et al, 2013” (S9 and HEP) and “Austin et al, 2002” (microsome) f_U calculation options.
- Composition of S9, microsome assays now determined by reported protein concentrations and lipid:protein and storage lipid: total lipid ratios.
- Updated HEP assay composition now based on liver composition, HEP cell concentration and spherical HEP cell volume calculation.
- Added compositional $f_{U, Assay}$ calculation option for S9, HEP and microsomal assays.
- Added full compositional details to body and blood for all IVIVE sheets; P_{BiW} now calculated using the compositional approach rather than Nichols et al regression.
- Updated default compositions and liver fraction for generic lab fish and lab fish blood.
- In vitro study reliability assessment DETs were changed so that a “low” reliability score is assigned if experimental data are estimated to not be significantly different than the simulated control.

IOCs

- Added partition coefficient scaling factor for the charged form for $K_{CarbohydrateW}$ ($10^{-3.5}$).
- Updated values for other scaling factors for the charged form for $K_{StorageLipidW}$ ($10^{-3.5}$) for acids and bases and K_{OC} ($10^{-1.3}$) for acids.
- Chemical activity ratios are not calculated for IOCs in the BAT, but they are in the BET.

Other revisions

- Updated density of membrane lipids, proteins and serum albumin as 1000 kg/m³, 1100 kg/m³ and 1360 kg/m³, respectively.
- Updated (BAFs and BCFs) lipid standardization to be lipid-normalized instead.
- Changed default selection of BAT-calculated wet-weight BAFs and BCFs selected values.
- Changed output to show BAT-calculated BAFs for planktivorous and piscivorous fish.
- Added a user option for showing the BAT-calculated BAFs and BCFs based on either total or freely-dissolved water concentrations.
- Changed BAT-calculated BAFs and BCFs for IOCs so they are always based on freely-dissolved water concentrations to address uncertainty in estimating IOC partitioning in bulk water.
- BAT-calculated terrestrial BMFs are now based on total ingestion (diet and water) rather than just the diet.
- Added column to the Results sheet showing units for each selected B-metric.

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- Moved chemical activity ratio output to end of the summary results table.
 - Updated field BAF/BMF and TMF study reliability assessment DET allowing users options for describing confidence in spatial and temporal consistency of data collection and the steady-state assumption.

Bug fixes

- Changes made after initial physical-chemical parameterization; now always updated on physical-chemical output sheet.
- Correction for reading in cells for re-parameterizing organism type half-lives upon re-opening of previously saved BAT excel file.
- Corrected organism and diet density calculation to use volume fractions rather than mass fractions.
- Input Lab BCF and Lab BMF study calculations updated to use organism compositions based on volumetric basis.
- Applied further limits on possible calculations to simulate input lab and field studies based on entered/missing study information.
- Chemical activity ratio output for neutral organic chemicals for BMFs reflect the composition of entire organism and diet, not only lipid.
- Addressed occasional errors for calculations/outputs for growth-corrected output checkbox.
- Addressed a few typos in the BAT user interface.