The Bioaccumulation Assessment Tool (BAT)

Version 2.02

QUICK START GUIDE

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QUICK START GUIDE

Using the Bioaccumulation Assessment Tool (BAT)

Updates in Ver.2.02 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

1. Open the BATver.2.02.xlsm file.
2. 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.
3. 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.
4. A dialogue box will open providing two options, i) Open Quick Start PDF and ii) Open BAT.
5. 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).

6. Select "Neutral" if the chemical is a neutral organic, or "Ionic" if the chemical is an ionizable organic chemical (IOC).
7. Identify yourself in the Assessor's Name and Organization textboxes (for display in the Report).
8. 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).
9. 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 9 for guidance on this step.
10. Click the "Initialize BAT" button.
11. 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.
12. 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).
13. 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 13-14 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) or click on the "Next" button to enter other bioaccumulation data (Step 4).

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.

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 spLFER, optional for ppLFER).
- The user can also enter other distribution ratios on the same tab of the user-entry form (see **User Manual** pages 16 – 18 for more information).

Follow steps 2 – 6 as for neutral chemicals above.

Step 3: Biotransformation

1. You can assume 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), or In Vivo biotransformation half-life data, or In Silico (QSAR) biotransformation half-life 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

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

In BAT Ver.2.02, 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.

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

8. Enter the organism type (type in fish or mammal).
9. Enter the relevant information from the in vivo study.
   - Source
   - Species
   - Biotransformation half-life (days)
   - Mass of organism (kg)
   - Temperature (°C)
10. 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.
11. Repeat as necessary to enter all available data and complete the associated DETs.
12. 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.
13. 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.
14. 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: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 (BAT-calc, Composition or"1") for fraction unbound in the test system using the drop-down menu. See **User Manual** pages 25-29 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/10^6 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 microsomal 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)

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 Biotransformation Scaling Factors

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

Step 5: Enter Bioaccumulation Data

1. Return to the **BAT Main** sheet and use the checkboxes to select 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 10 generic B-metrics (see **User Manual** pages 51-55 and **Appendix A7**) using the provided property and biotransformation data.
3. Select the type of Bioaccumulation Data 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)
   - 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)
   - 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.

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)
   - 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)
   - 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.

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 (µg/kg)
     - Concentration of chemical in water (total, µg/L) OR
     - Concentration of chemical in water (dissolved, µg/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 (µg/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)
     - Concentration of chemical in diet/prey (µg/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, µg/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 (µg/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 **Summary Results** 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 bult-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.
     - **Fugacity ratios** (if applicable, e.g., not applicable to IOCs) for each LoE.
     - 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.
     - 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. **Report PDF**: The Report PDF 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 HLAW_T(medium) now correctly output.

Updates to built-in BAT model 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:

☑ Added an option to enter chemical-specific enthalpies of phase change to improve temperature correction of partitioning behaviour.
☑ Increased capacity to enter up to 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; a "pop up" for guidance facilitating this process is also now included.

- ☑ Added chemical degradation in the gastro-intestinal tract (GIT) estimated from entry of Dietary Absorption Efficiency (E_D) study entry sheet.
- ☑ Added invertebrate BCF LoE study entry sheet and DET form.
- ☑ Added rodent TK BMF LoE study entry sheet and DET form.
- ☑ Added organism type and/or species identification as required input to study LoE.
- ☑ If the field study LoE, e.g., BMF, is known, it can now be entered directly without adding the data necessary to calculate it.
- ☑ Updated in vitro and in vivo data reliability forms and scoring to be consistent with associated databases included in EASE-Suite.
- ☑ In vitro biotransformation rate assay composition is now automatically calculated based on reported protein concentrations; these can also be overwritten by the user, if desired.
- ☑ Added fugacity ratio calculations 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 B 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 included in the assessment outcome results.
- ☑ Output for generic lab invertebrate BCF, BMF and lab rat BMF are integrated into **Summary Results.**
- ☑ Output for generic field aquatic mammal ("Seal") BMF are integrated into **Summary Results.**
- ☑ 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).
- ☑ New output for the total half-life of each output B-metric/organism on a new output sheet "HLT".

Updates to the built-in BAT model calculations:

- ☑ Dietary absorption efficiency (E_D) model calculation updated to Arnot and Mackay 2018.
- ☑ Revised bioenergetics and water mass balances for aquatic and benthic invertebrates in aquatic food web.
- ☑ Added an aquatic mammal (seal) as a top predator in the aquatic food web.
- ☑ Added separate laboratory conditions for aquatic invertebrates, fish, and rat.
- ☑ Updated fugacity ratio calculations.
- ☑ If multiple biotransformation HL values for each organism type (e.g., "fish" and "mammal") are available, a weighted geometric mean of the biotransformation HLs 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.
- ☑ 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.

☑ Temperature corrections to environmental and biological partition coefficients for neutral and ionic chemicals occur throughout the BAT based on the built-in BAT model (BAT-Estimated) and reported (study) organism and environmental temperature conditions.

☑ Added the "compositional approach" for estimating f_u as a user option for extrapolating in vitro biotransformation rate values to hepatic clearance and whole-body level values.

☑ Correction in BAT Ver.2.0: Zdiet for mammals consuming fish now considers both the neutral form and charged form of IOCs.

Updates in BAT Ver.2.02

☑ User-entered studies (LoE) are now only included for the **Strength of Evidence** (SoE) summary table, *IF the LoE has a reliability score (RS) > 0*. For transparency, if a user-entered LoE has a RS = 0, the data are still displayed in the **Summary Results** Sheet.

☑ Updated default parameters for lab system temperature, fish growth rates, and dietary composition for the built-in BAT model calculations to reflect median values from experimental bioaccumulation testing databases (Arnot and Quinn 2015, Arnot and Gobas 2006).

☑ Fish bioenergetics equations now differentiate between lab and field conditions (temperature).

☑ Addition of user-defined weighting of biotransformation study types to the **Chemical Summary** Sheet. These user-define values are then used in the weighted geometric mean HL_B values used by the built-in B models. Defaults are provided if the user does not choose their own values.

☑ Addition of a user-defined option for scaling fish biotransformation rate constants to invertebrate biotransformation rate constants to parameterize the built-in BAT models. Otherwise, the default assumption for invertebrate biotransformation is 3 times the fish biotransformation HL.

☑ Addition of a user-selected option for using growth corrected B metrics from lab studies

☑ Corrections:

- Extra "theta" removed from BAF_{SS} calculation (input was updated to use dissolved fraction for concentration in January 2021), because "*theta" was redundant in these calculations.
- Steady-state and Kinetic lab B-metric calculations provided by the built-in BAT model are now consistent / aligned.
- HLT output erroneously appeared in units of hours but it was labelled days; output units now corrected to days.