ICE IVIVE Tool

Contents

Introduction

The ICE IVIVE (In Vitro to In Vivo Extrapolation) tool allows you to estimate the equivalent administered dose (EAD) that would result in the plasma concentration of a chemical equal to the activity concentration in a given in vitro assay. The tool also lets you graphically compare EAD results with specific types of in vivo data or predicted exposures from the U.S. Environmental Protection Agency (EPA) SEEM3 model (Ring et al. 2019) that are available in ICE. You can also compare EAD results with in vivo or exposure data you upload to the tool.

You can run the IVIVE tool interactively via the ICE graphical user interface with inputs from ICE data or your own in vitro assay data. You can also download the workflow as an R notebook to run IVIVE analyses locally.

Located throughout the tool are green information buttons to help you better understand key features and results. When you hover over a button, brief explanatory text will appear. When you click a button, more details will appear in a text box that can be resized and relocated on the screen.

The “Help” button on the left side of the display opens a text box with a brief description of the tool and links to the webpage and downloadable user guides. Below this, clicking on the “Help Video” button will provide access to videos demonstrating how to build a query and evaluate results. If you encounter a problem using a tool, click the “Report an Issue” button below the “Help” button to generate an email to ICE Support.

Building an IVIVE Query

Figure 1 shows the ICE IVIVE tool Input view. You can toggle between Input view and Results view by clicking tabs on the left side of the screen. The tool window defaults to Input view when it is first opened.

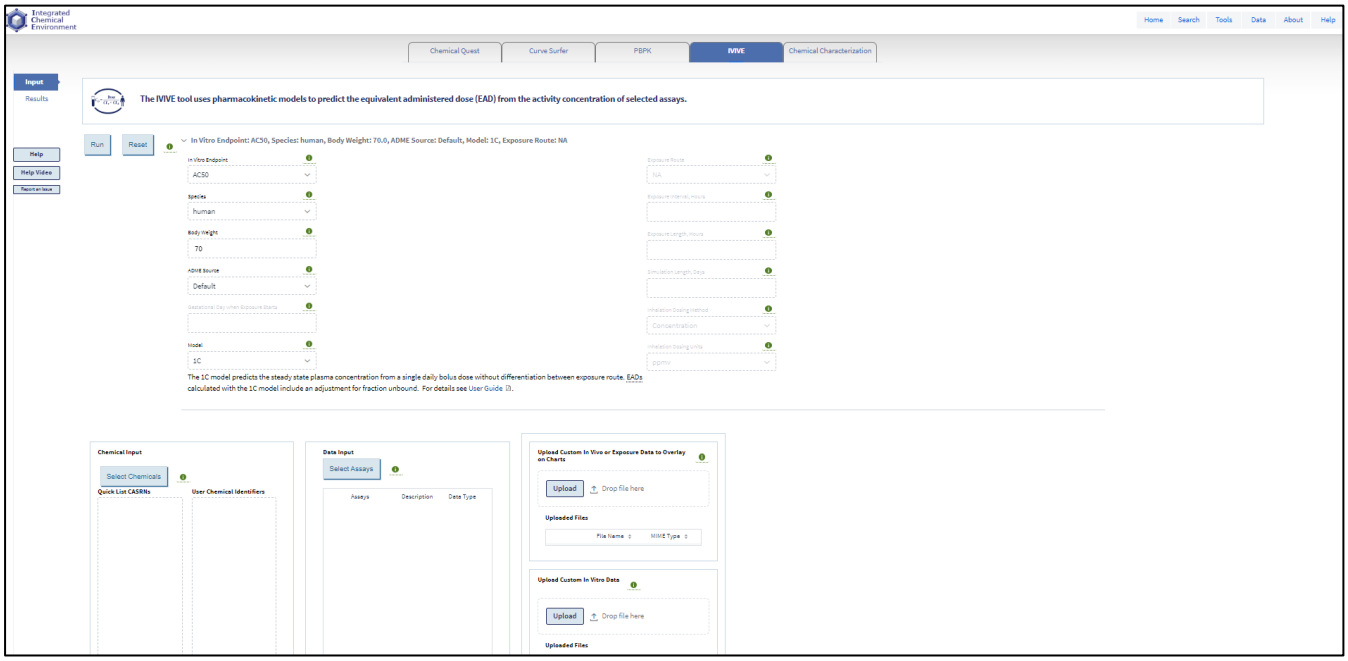


Figure 1. ICE IVIVE tool Input view. [Screenshot from ICE 4.0.2, updated 1 Feb 2024.]

To run the IVIVE tool, you must input both assays and chemicals. You must also select an in vitro endpoint, species, and pharmacokinetic model, and specify dosing parameters. Default settings are provided and are shown in **Figure 2**. Users can provide their own in vitro assay endpoint data for IVIVE analysis and their own in vivo or exposure data to view in comparison.

Specify Endpoint, PK Model, and Model Parameters

The IVIVE tool allows you to select one of five pharmacokinetic (PK) models for calculating an EAD. For all models, you must specify an in vitro data endpoint, a species, body weight and the source for model parameters. Multiple-compartment physiologically based PK (PBPK) models also allow you to select exposure route, exposure interval, and simulation length. You will also need to specify exposure length, and inhalation dosing method and units for the inhalation model, or gestation day on which exposure starts for the pregnancy model. Click the dropdown lists above the Chemical and Data Input fields to select PK models and dosing parameters for your query (**Figure 2**).

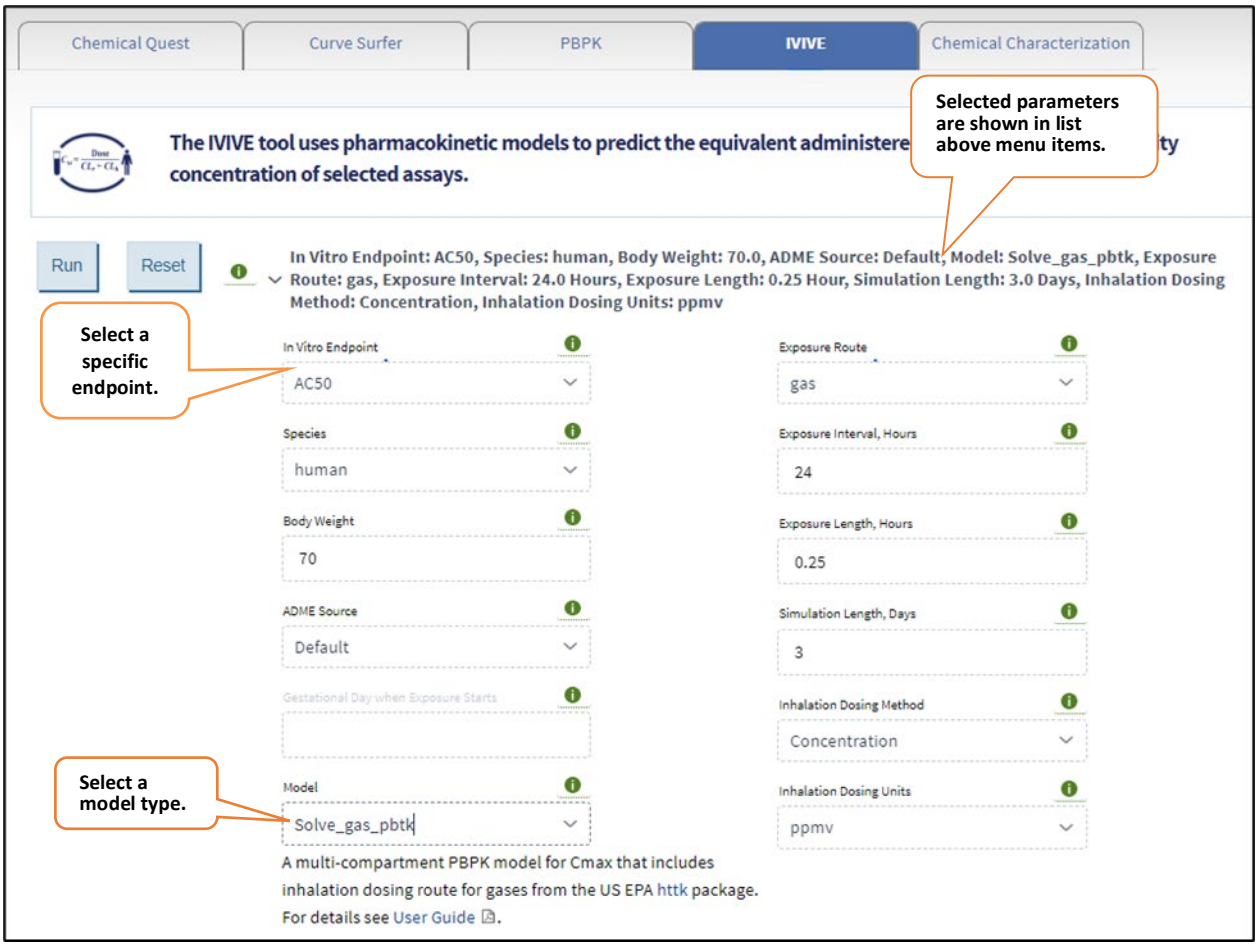


Figure 2. Click dropdown lists to specify endpoint, model, and model parameters. [Screenshot from ICE 4.0.2, updated 1 Feb 2024.]

Select In Vitro Endpoint

Specify an in vitro endpoint value for the ICE curated high-throughput screening (cHTS) data that will be used:

- Half-maximal activity concentration (AC50) (default).
- Activity concentration at cutoff (ACC).

This selection is not needed if you are uploading custom in vitro data (see "Option to Upload Custom Data" below).

Enter Body Weight

Enter the body weight of the simulated subject in kilogram (kg). The default body weight is 70 kg for human and 0.25 kg for rat.

Select Species

Select the target species that the tool will create predictions for. Currently, selections are limited to rat and human (default). For certain PK parameters, if rat values are not available, human values are

substituted with proper allometric scaling.

Select ADME Source

Select the source of the absorption, distribution, metabolism, and excretion (ADME) data (intrinsic clearance and fraction unbound) to use in modeling.

- **Default:** experimentally measured values will be used when available. If a measured value is not available, an in silico prediction from the Open Structure–activity/property Relationship App (OPERA) will be used.
- **Measured:** only experimentally measured values will be used; this option may result in fewer predictions.
- **In Silico:** only in silico predictions from OPERA will be used.

Select Model

Select one of five PK models:

- **1C (default):** a single-compartment, population-based PK model. This model assumes 100% absorption after a daily bolus dose (equivalent to the intravenous [IV] exposure route). It uses a Monte Carlo-simulated population to account for inter-individual physiological variations (Wetmore et al. 2012). It estimates steady-state plasma concentration (Css) representing the 50th percentile (median) and upper 95th percentile of the distribution. The parameters for executing the 1C model are provided by ICE. This model generates an EAD that provides the unbound plasma chemical concentration at steady state equal to the in vitro activity concentration.
- **Solve_3comp:** a three-compartment model from the EPA high-throughput toxicokinetics (httk) R package (httk v 2.2.2). The model includes gut, liver, and rest-of-body compartments. The model uses the httk "Solve_3comp" function to estimate maximum concentration (Cmax) in plasma, based on which a median (50th percentile) EAD value corresponding to in vitro activity concentration is estimated. It can simulate the oral or IV injection route. The parameters for executing this model are provided by ICE.
- **Solve_pbtk:** a multiple-compartment PBPK model from httk v 2.2.2. The model includes gut, artery, vein, lung, liver, kidney and rest-of-body compartments. The model uses the httk "Solve_pbtk" function to estimate plasma Cmax, based on which a median EAD value corresponding to in vitro activity concentration is estimated. It can simulate the oral or IV injection route. The parameters for executing this model are provided by ICE or by httk v 2.2.2.
- **Solve_gas_pbtk:** a multiple-compartment PBPK model from httk v.2.2.2. The model includes the same compartments as the “Solve_pbtk” model but has an additional dosing module for the gas exposure route.
- **Solve_fetal_pbtk:** a multiple-compartment PBPK model from httk v 2.2.2 that includes maternal compartments, fetal compartments, and a placenta modeled as a joint organ shared by mother and fetus. It predicts maternal and fetal chemical distribution for

exposure via the oral or IV injection route starting at 91 to 280 days' gestation. This model is for human only.

You can find more information on all five PK/PBPK models, including how additional input parameters are obtained, in Appendix 1.

Enter Gestation Day

Enter the gestation day when exposure starts. The range of possible values is 91 to 280 days, with the default set to 91 days (i.e., 13 weeks). This parameter is only used for Solve_fetal_pbtk model.

Select Exposure Route

Select the route of administration that the PK/PBPK model will simulate. Options are specific to the model selected; not all routes will be available for all models. They include oral, IV injection, and gas (inhalation).

Enter Exposure Interval

Enter the dosing interval in hours that the model will simulate. The parameter defaults to 24 hours, equivalent to one dose per day. This parameter is not used for the 1C model.

- ICE now allows you to compare EADs with population-level human exposure predictions from EPA's SEEM3 model. Best results for this visualization option are obtained by using the default exposure interval of 24 hours.

Enter Exposure Length

Enter the length of the exposure in hours for the inhalation exposure route. The parameter defaults to 0.25 hours, equivalent to a 15-minute exposure. This parameter is only used for the Solve_gas_pbtk model.

Enter Simulation Length

Enter the exposure duration in days that the PK/PBPK model will simulate. The parameter defaults to "3" for a three-day exposure. Dosing will occur at the frequency specified by the Exposure Interval field for the duration of the simulation.

Select Inhalation Dosing Method

Select how the exposure is to be modeled. The only option currently available is "Concentration," which models the concentration of the chemical in air as either parts per million by volume (ppmv) or µM for the duration of the exposure. This parameter is only used for the Solve_gas_pbtk model.

Select Inhalation Dosing Units

Select whether ppmv (default) or µM will be used for chemical concentration for gas exposure. This parameter is only used for the Solve_gas_pbtk model.

Chemical Input

In the Chemical Input field, add chemicals to your query using one or both input methods (**Figure 3**):

- To populate the left-hand text box, select one or more ICE Chemical Quick Lists by clicking the "Select Chemicals" button. In the dialog box that opens, select the checkboxes to choose one or more chemical lists. Click "Finished" when you are done. Chemical Abstracts Service Registry Numbers (CASRNs) from the selected ICE Chemical Quick Lists will populate the Quick List CASRNs text box.
    - While in the dialog box, you can download ICE Chemical Quick Lists by clicking on the download icons to the right of the list names.
    - Each download provides the list of chemicals along with additional metadata relevant to the Chemical Quick List.
- To populate the right-hand text box, enter your own list of chemical identifiers (one per row) in the "User Chemical Identifiers" text box. You can use any combination of the following identifiers: CASRNs, chemical name, Distributed Structure-Searchable Toxicity Substance Identifiers (DTXSIDs), Simplified Molecular-Input Line-Entry System (SMILES) strings, or Hashed International Chemicals Identifiers (InChIKeys).

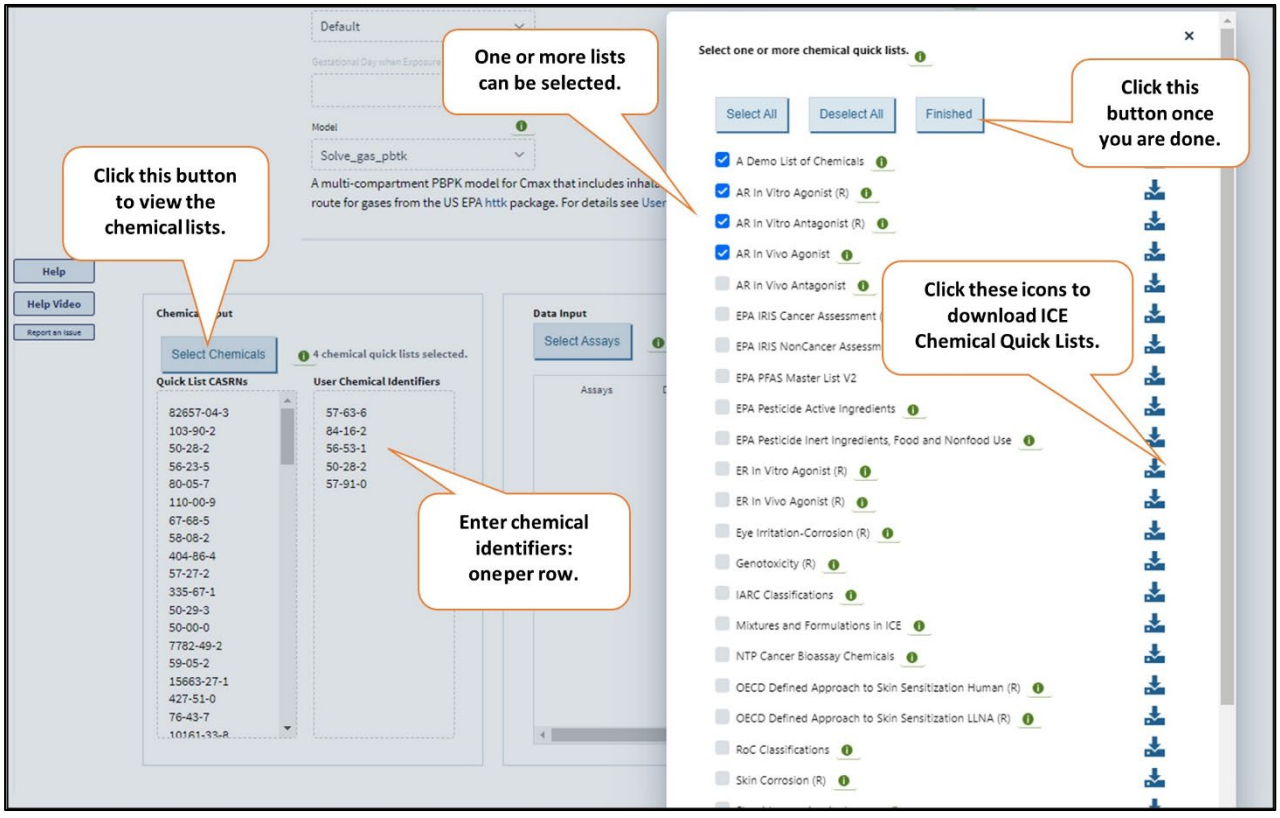


Figure 3. Input chemicals by typing chemical identifiers into the text box (right background) and/or selecting ICE Chemical Quick Lists (left background). Dialog box for selecting Chemical Quick Lists is in the foreground. [Screenshot from ICE 4.1, updated Aug 2024.]

Note the following limitations:

- Only chemicals with cHTS data in ICE can be used to build IVIVE predictions unless you provide custom in vitro data (see "Option to Upload Custom Data" below).
- Only chemicals with the required PK parameters for running the IVIVE analysis available in ICE will be used to build IVIVE predictions. This is not a limitation in most cases as ICE includes parameter predictions from OPERA for over 800,000 chemicals.

Data Input

The Data Input panel, located below the model parameters on the right (**Figure 4**), allows you to input in vitro assay data for specific toxicity endpoints orcategories of interest. Click the "Select Assays" button, which will open a dialog box offering two options for selecting assays.

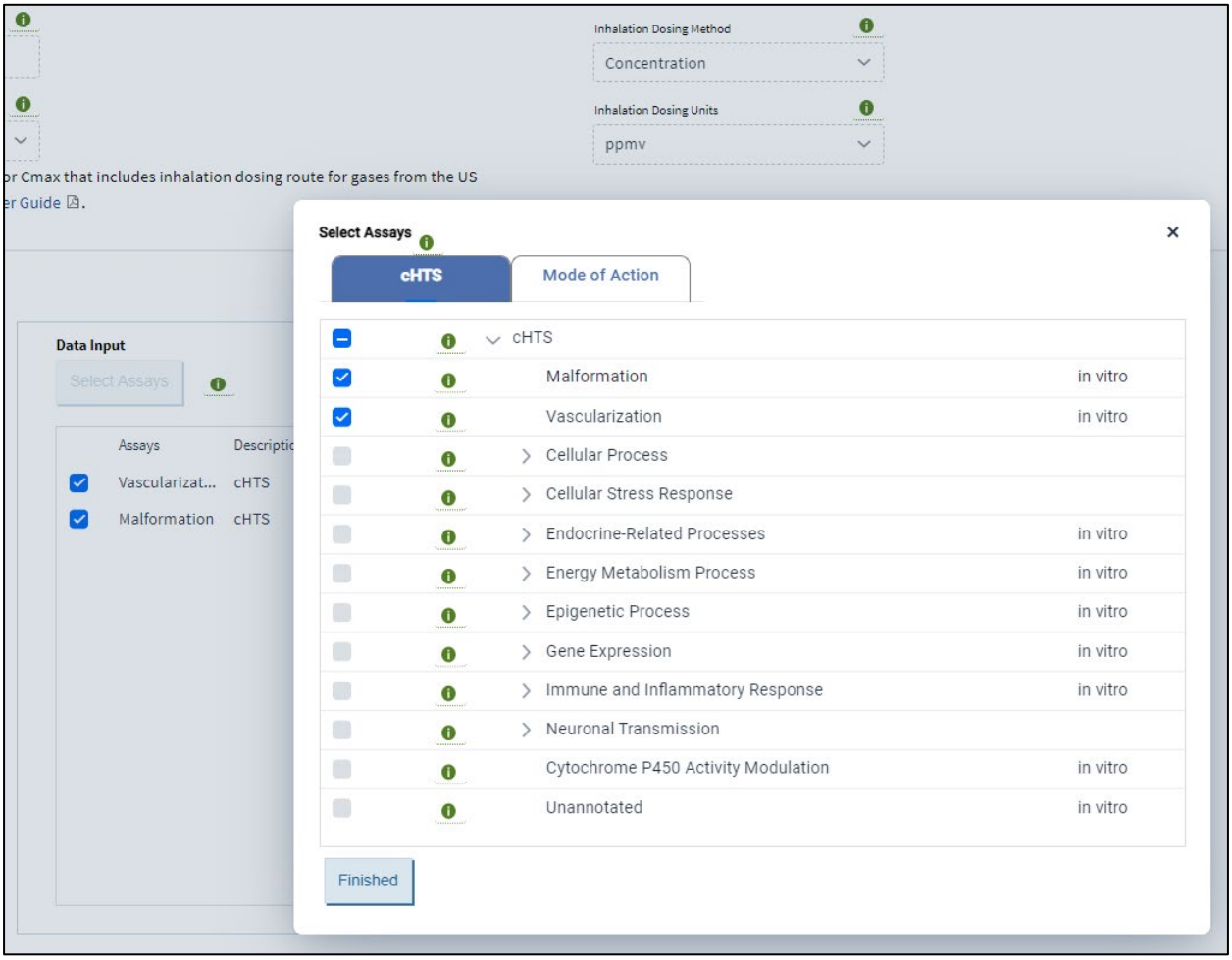


Figure 4. Specify assays to use in the tool. [Screenshot from ICE 4.0.2, updated 1 Feb 2024.]

- Under the "cHTS" tab is a list of high-throughput assays organized by mechanistic target terms. Options within this category are limited to cHTS data in ICE.
- Under the "Mode of Action" tab assays are organized by mode of action relevant to toxicity endpoints of regulatory concern. Note that if a Mode of Action is selected, the results will show all mode of action annotation information for each selected cHTS assay for the specific toxicity endpoint that mode of action is under. This means that additional modes of action

may appear in the results for the specified toxicity endpoint depending on what assays were selected. You will be able to examine data from individual assays by filtering on the assay name in the Results table when query is completed.

Select the checkboxes to select assays under either one or both tabs. For more details on the assays and groupings, see the "cHTS" section of the ICE Data Sets page.

Option to Upload Custom Data

Instead of or in addition to using cHTS data in ICE as input for the IVIVE models, you can supply your own in vitro assay endpoint data to be used as input for your model. Note that only chemicals with parameter values available in ICE will be modeled. You can also provide your own in vivo assay or exposure data to compare with predicted EADs in the Results view. These options are provided in the Upload Custom Data fields on the right-hand side of the Input view **(Figure 5)**.

Before you upload any data, they must be properly structured and formatted. The green information button to the left of each of the Upload Custom Data field headings opens a dialog box that provides links to text and Excel templates for data files.

- For in vivo or exposure data, you will need to provide:
    - Chemical identifiers. There are three fields for CASRNs, DTXSIDs, and chemical names. You must provide a CASRN for each chemical, and you must populate all three fields. If you do not have a DTXSID or a chemical name for a chemical, populate the field with "NA".
    - Data set identifiers. Used to distinguish different types of data; for example, data from different kinds of in vivo assays. Any text string of 20 characters or less is acceptable.
    - Assay data. Numerical data only for the relevant assay. Units, while not specified in the cells, should align to those for EAD values estimated based on the PK model type and dosing regimen you have selected for your prediction. Specifically, data should be in units of mg/kg/day for the 1C model; mg/kg/dose for the Solve_3comp, Solve_pbtk and Solve_fetal_ pbtk models; and ppmv or µM/dose for the Solve_gas_pbtk model. Note that "mg/kg/day" is equivalent to "mg/kg/dose" if you select a dosing interval of 24 hours.
- For in vitro data, you will need to provide:
    - Chemical identifiers as CASRNs.
    - Data set identifiers to distinguish different types of in vitro assay data. Any textstring of 20 characters or less is acceptable.
    - Assay data: the activity concentration (concentration at which a response is observed in the assay) in µM units.

Once you have created your data file, you can drag and drop the file into the appropriate input field or click the "Upload" button to open a file browser dialog box. After your file is uploaded:

- Names of uploaded files will be displayed in a list below the “Upload” button in the appropriate field.
- In vivo or exposure data will not be displayed in Input view but can be viewed in the Results view graphs.
- CASRNs for chemicals in your uploaded in vitro data set will be displayed under the User Chemical Identifiers section of the Chemical Input field, one identifier per line.
- Information for assays in your uploaded in vitro data set will be displayed in the Data Input box.

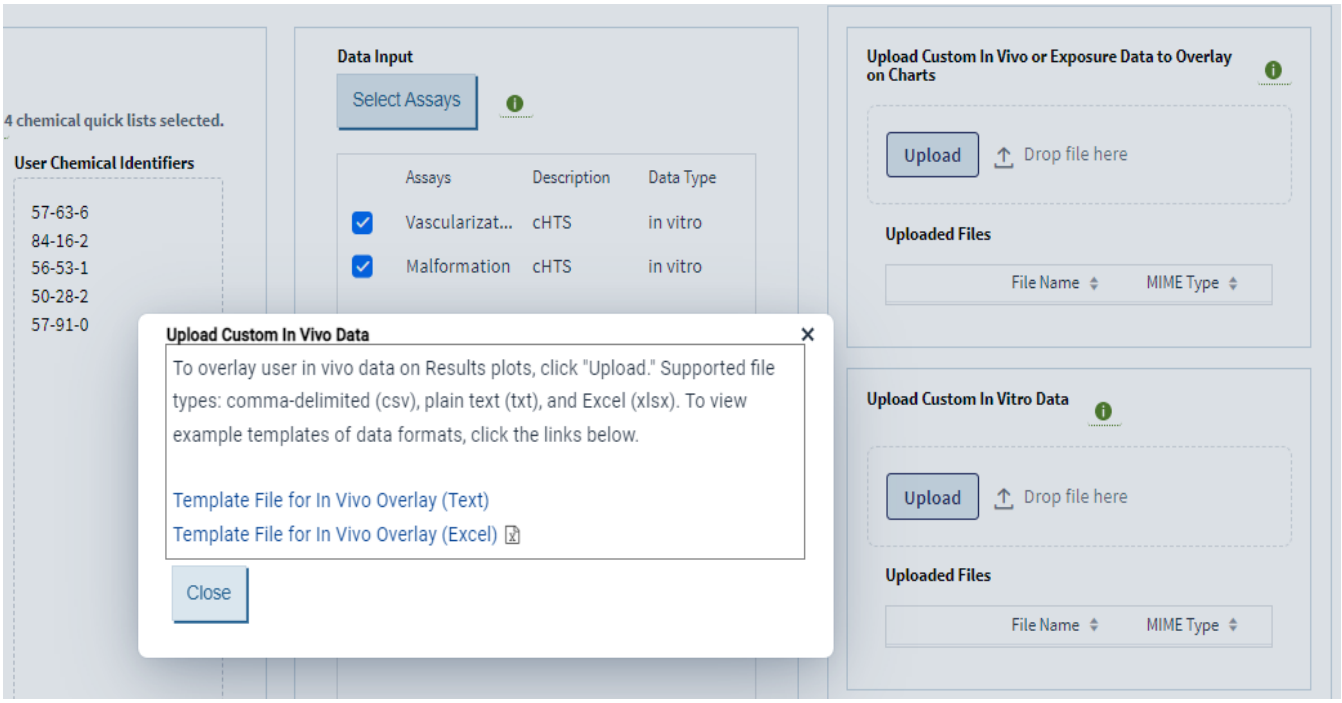


Figure 5. Upload your custom in vivo, in vitro, or exposure data. [Screenshot from ICE 4.0.2, updated 1 Feb 2024.]

Run IVIVE Tool

Once you have imported or selected assays, chemicals, and other parameters, click on "Run" at the top left of the page to run your query (**Figure 6**). The query may take a few minutes, depending on the model and inputs. To clear all inputs, select "Reset".

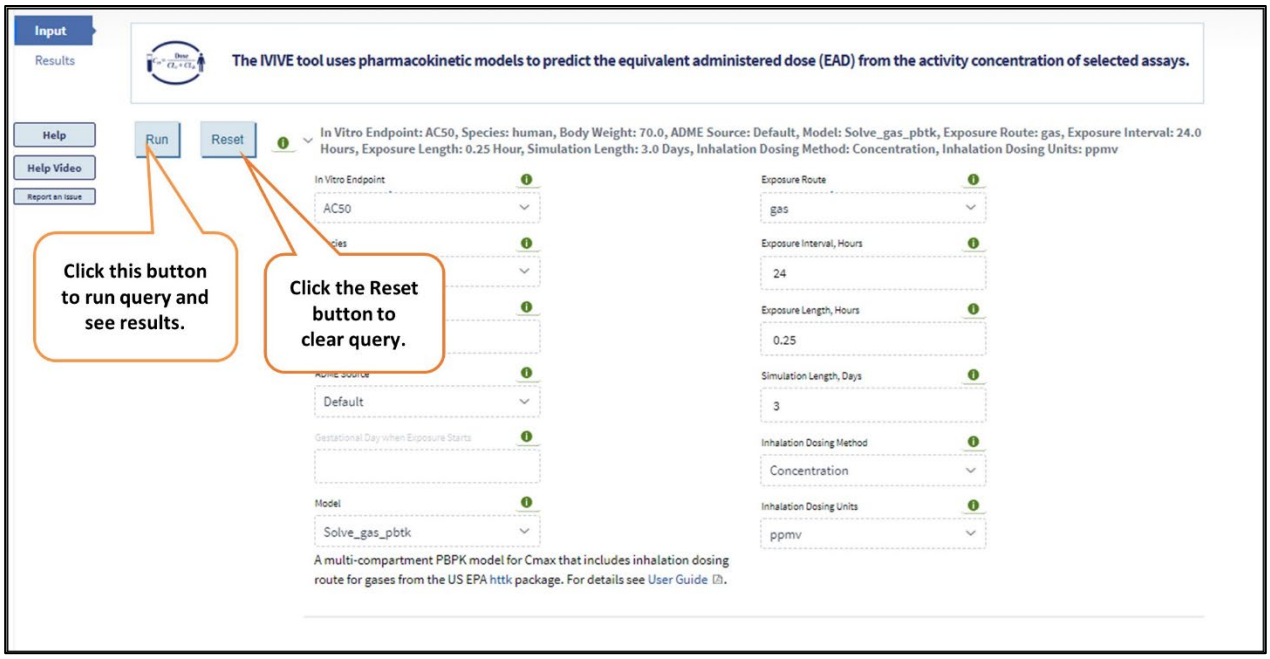


Figure 6. Run IVIVE tool. [Screenshot from ICE 4.0.2, updated 1 Feb 2024.]

Viewing IVIVE Results

The window will switch to Results view when the query is run (**Figure 7**). If no relevant data were found for any of your query chemicals, a "No data returned from query" message will appear. Click “Input” in the top left to return to Input view to review or change your query parameters and rerun your query.

Download Results

At the top of the Results view, click on “Download IVIVE Files” to view a link to the default output file, “Download IVIVE Results.” This is an Excel workbook that contains two different worksheets.

- The first worksheet, “EADResults”, contains the model parameters and the EAD calculations. For the 1C model, EAD.50 and EAD.95 are both returned, while for other models, only EAD.50 is returned. The solve_fetal_PBTK model returns both an EAD.50 value, which corresponds to maternal plasma Cmax, and an EAD.fmax.50 value which corresponds to the fetal plasma Cmax. You will need to scroll to the right of the empty “EAD.95” columns to view the EAD.fmax.50th values. A “Flag” column is included by default in the event any warnings are returned for the chemical (described below).
- The second worksheet, “inVitroData”, contains the in vitro data used in the EAD calculations.

Interactive Results View

Below the download file link, your query results are displayed in an interactive table and two interactive graphs (**Figure 7**). Icons above the table provide options to export the table data to either a text file or an Excel file. Two graphs below the table display the daily EAD and the in vitro bioactivity (AC50 or ACC).

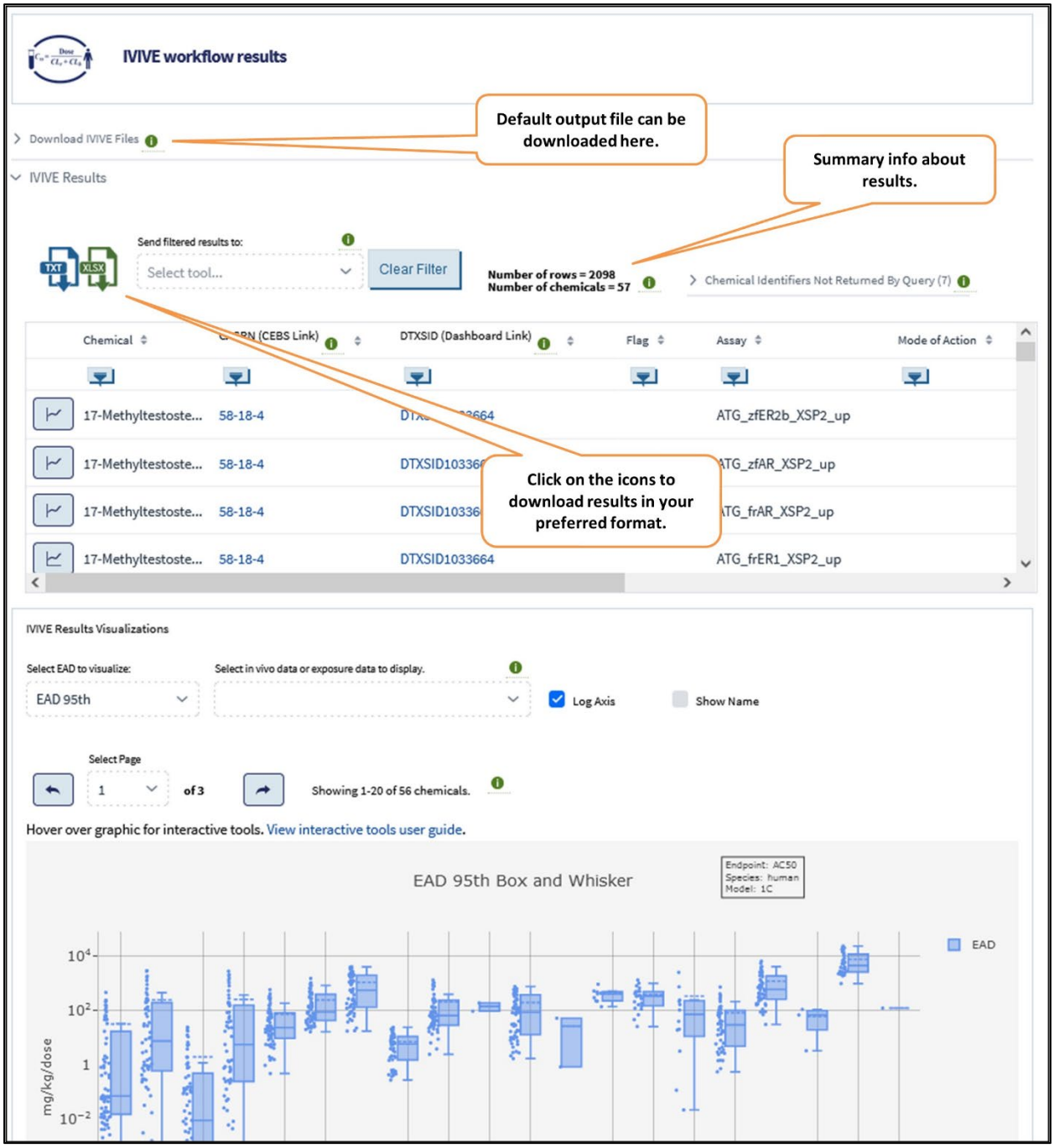


Figure 7. ICE IVIVE tool Results view. [Screenshot from ICE 4.0, updated 02 May 2023.]

Results Table

To the right of the download icons and dropdown list **(Figure 7)**, the number of rows and chemicals in the table are listed. There may also be a clickable heading labeled “Chemical Identifiers Not Returned By Query”. This provides a list of any chemical identifiers in your query with no relevant data in ICE for IVIVE. If no relevant data were found for any of your query chemicals, this will be the only output. A link below the list allows you to download the list as a text file.

The first column of the table contains a button that sends data from that row to the ICE Curve Surfer

tool **(Figure 8**). Clicking this button will display the activity curve for that chemical–assay combination. Refer to the Curve Surfer User Guide for details on this tool.

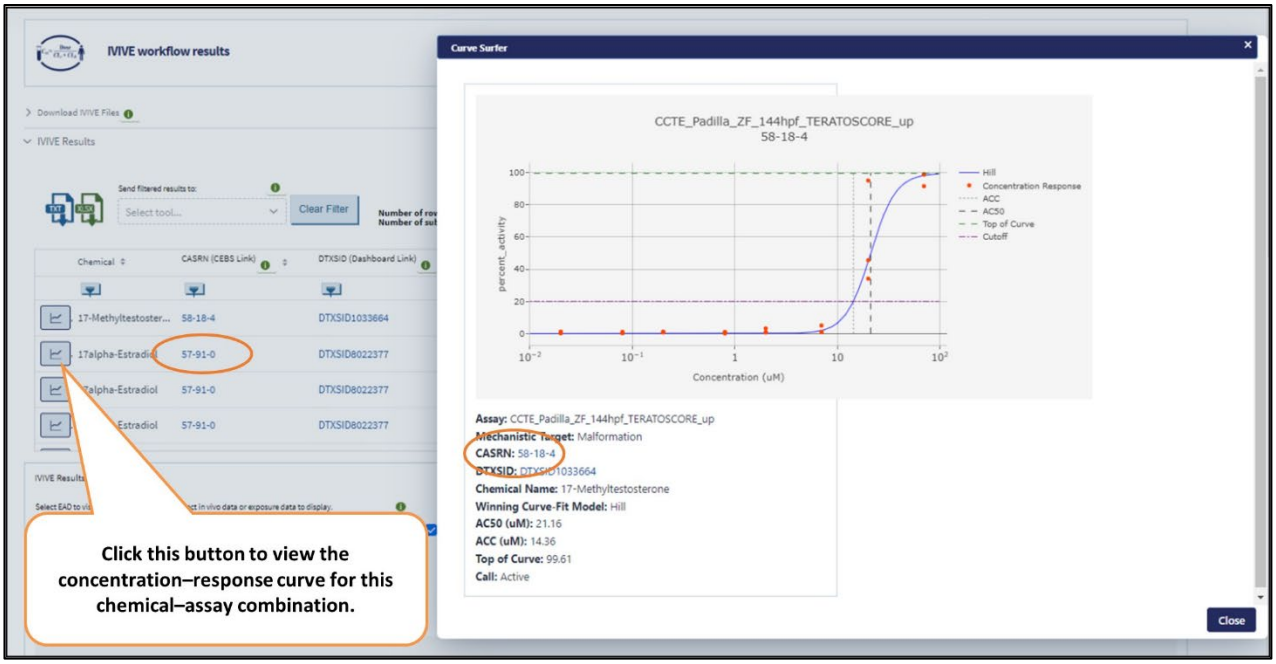


Figure 8. Curve Surfer assay data view. [Screenshot from ICE 4.0, updated 14 Apr 2023.]

The next three columns contain identifier information for each chemical.

- The first of these contains the chemical name.
- In the next column, click on the chemical's CASRN to be directed to the test article page about that chemical in the National Toxicology Program's Chemical Effects in Biological Systems (CEBS) database.
- In the third column, click on the chemical's DTXSID to be directed to the EPA CompTox Chemicals Dashboard entry for that chemical, which provides the chemical structure and other physicochemical and experimental properties.

To filter results on a specific chemical or assay, click the filter icon below the column heading and select items of interest in the dialog box that opens (**Figure 9**). The "Number of rows" and "Number of chemicals" listed above the table will be updated to reflect the filtered results, and the icon for the applied filter will turn red. The graphs below the table will be updated based on the filtered results. Clear filters by clicking the "Clear Filter" button above the table.

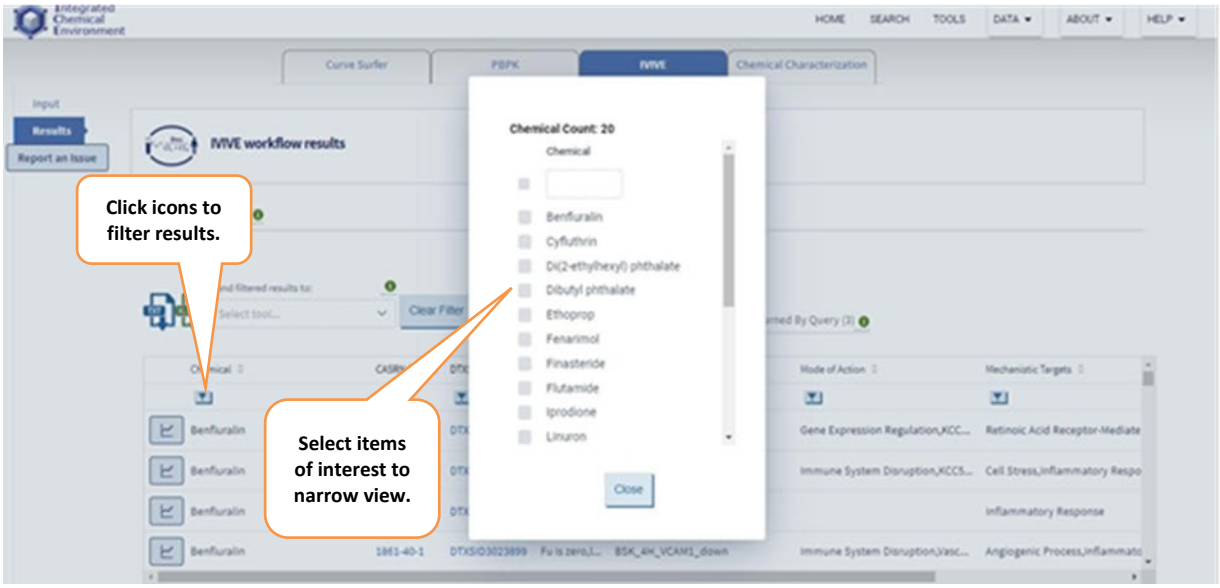


Figure 9. Filtering results. [Screenshot from ICE 4.0, updated 31 Mar 2023.]

Depending on the model parameters and the input chemicals, warnings may be issued about the assay results. These are provided in the "Flag" column.

- **Too low fraction unbound:** a warning of "Fraction of chemical unbound to plasma protein (Fu) is zero, likely due to rounding. Setting to 1e-5" is issued if the fraction unbound is less than or equal to 1e-5.
- **Chemical likely nonvolatile:** a warning of "Chemical likely nonvolatile, consider appropriateness of model" is issued if the solve_gas_PBTK model was used and the chemical fails the httk package check of the Henry's Law constant being less than or equal to that of glycerol. The solve_gas_PBTK model requires that a chemical be volatile and able to be taken up in the gas phase.
- **Gestation day out of range:** A warning of "The gestation day when exposure starts shall be within the range of 91-280 days" is issued if the gestation day when dosing starts entered by user is out of the range of 91-280 days.

The remaining columns provide assay information and the assay results.

To download and save the table data, click the download icons above the results table to export eithera text file or an Excel file. Any filters applied to the displayed data will also be applied to the download.

Interactive Plots

The graphs below the results table display two interactive plots (**Figure 10**). Any filters applied to the interactive results table will also be applied to the graphs.

The upper plot shows a box-and-whisker plot of EAD results. The left-hand dropdown menu above the plot allows you to select data visualization options for the EAD results (**Figure 10**).

- If you chose the 1C model, the plot will display EAD values in blue. Click the "Select EAD to visualize" dropdown list above the left side of the graph to view either the EAD 50th or EAD 95th, which are EAD values corresponding to the 50th or upper 95th percentile values in the plasma Css distribution.
- If you chose the Solve_fetal_pbtk model, the "Select EAD to visualize" dropdown list gives you the option of viewing the EAD 50th, corresponding to the 50th percentile maternal plasma Cmax, or the EAD.Fmax.50th value, corresponding to the 50th percentile fetal plasma Cmax.
- If you chose any of the other models, the only display option will be the EAD 50th, corresponding to the 50th percentile plasma Cmax.

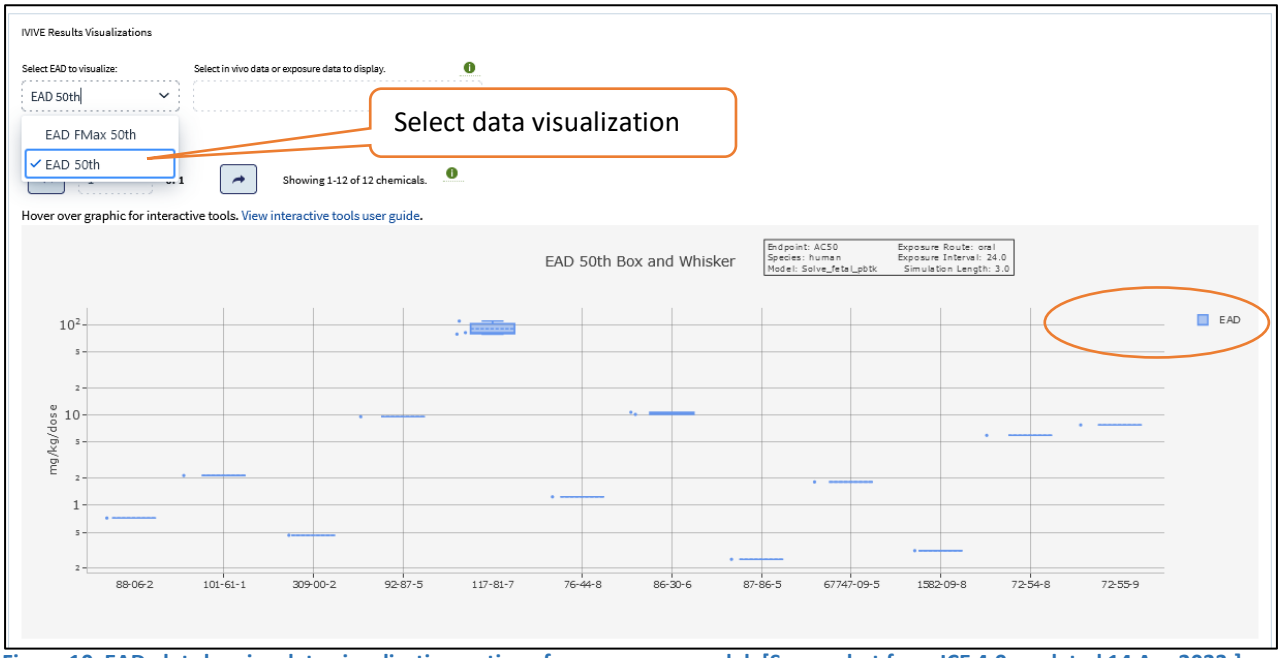


Figure 10. EAD plot showing data visualization options for pregnancy model. [Screenshot from ICE 4.0, updated 14 Apr 2023.]

To the right, the "Select in vivo data or exposure to display" dropdown list allows you to select in vivo data or Exposure Predictions to compare to your returned EAD values, which will appear in orange (**Figure 11**). Options provided by ICE include:

- Estrogen Modulation (Uterotrophic LEL [lowest effect level], mg/kg/day).
- Acute Lethality (Acute Oral Toxicity Assay LD50, [median lethal dose], mg/kg/day).
- Androgen Modulation (Hershberger, rat agonist LEL, mg/kg/day).
- Androgen Modulation (Hershberger, rat antagonist LEL, mg/kg/day).
- Exposure Predictions (Daily human exposure, mg/kg/day, predicted from EPA's SEEM3 model. Presented as the 5th, 50th, and 95th percentile of exposure).

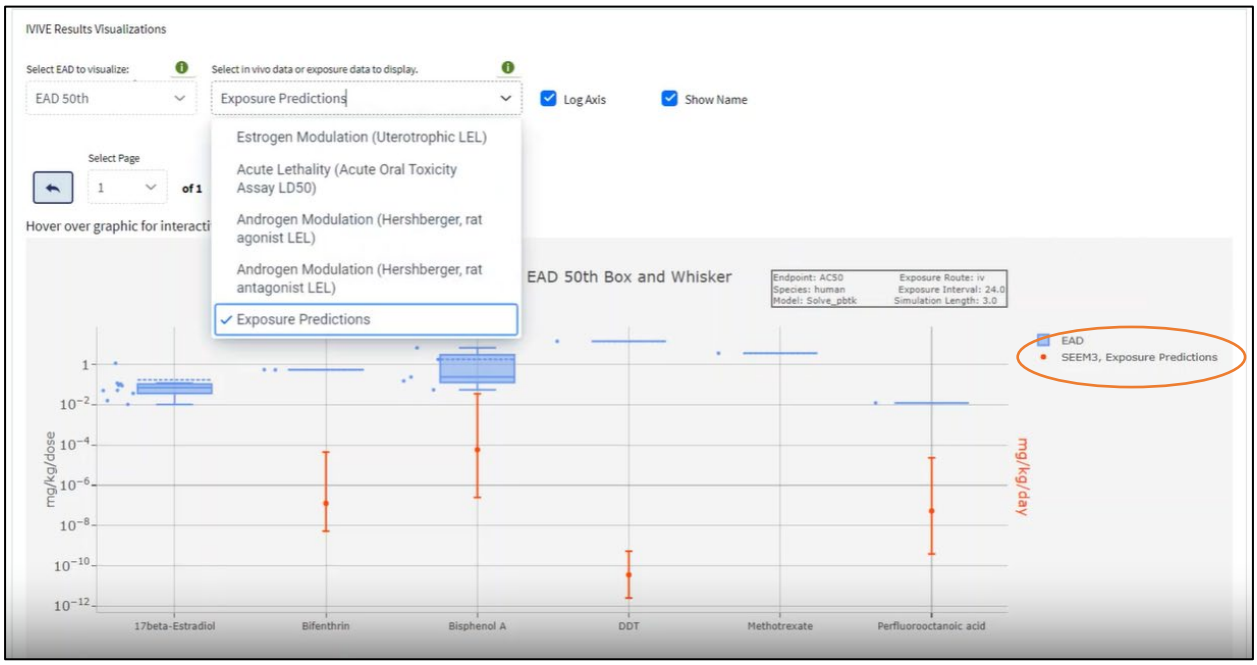


Figure 11. EAD plot showing options for in vivo or exposure predictions data comparisons. [Screenshot from ICE 4.0.2, updated 1 Feb 2024.]

Note that in vivo data and exposure predictions are based on a daily exposure, which best matches EADs that are generated from PK models with a dosing interval of 24 hours.

- We strongly suggest using an exposure interval of 24 hours when applying the exposure predictions to the display to ensure appropriate comparisons to EADs.
- We also recommend not comparing exposure predictions with EADs generated for the inhalation exposure route, as those are not directly comparable to assumptions for exposure predictions.

If you uploaded your own in vivo or exposure data, you will also have the option to select these from the in vivo and exposure prediction data sets to overlay on the plot. The labels for your user-provided data will be the ones you specified in the “Data set” column of the upload file.

The lower graph shows a violin plot of the in vitro bioactivity input values as either ACC or AC50, depending on your input (**Figure 12**).

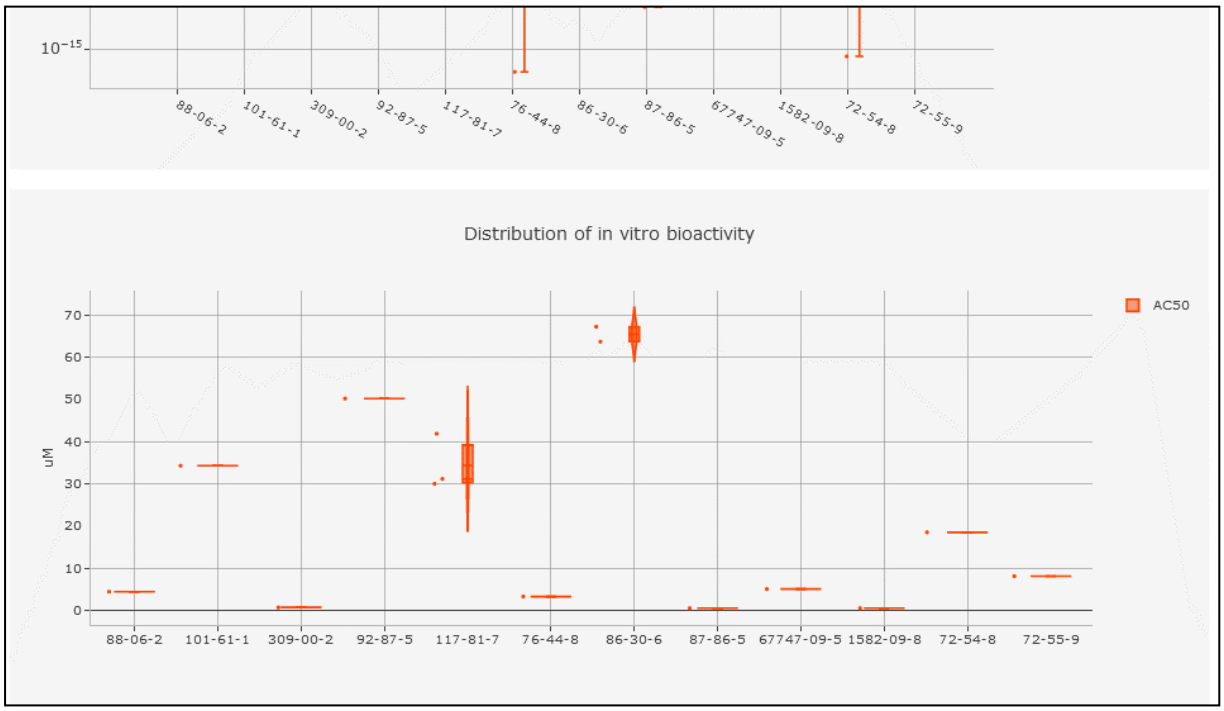


Figure 12. Violin plot showing range of AC50 values. [Screenshot from ICE 4.0, updated 14 Apr 2023.]

Two checkboxes to the right of the dropdown lists above the EAD plot allow you to select plot display options (**Figure 13**).

- The "Log Axis" box is checked by default. Unchecking it will change the y-axis on the EAD plot from log to conventional scale.
- Checking "Show Name" will change the x-axis labels on both plots from CASRNs to chemical names.

Hover over either plot area to display a menu of tools in the top right corner that can be used to adjust the graph display. Hover over the data bars in the graph to display summary statistics of the data for each bar. Click on an in vitro assay data point to view the cHTS activity curve for that chemical–assay combination. To zoom in on specific data points, click and drag the cursor over the area of interest on the graph, or use the toolbar in the top right of the plot area. Double-click on the plot to zoom back out. For more information about interactive graph visualization options, consult the Interactive Graphs User Guide.

The x axis of each plot is limited to displaying 20 chemicals; use the "Select Page" dropdown and buttons to select or browse pages.

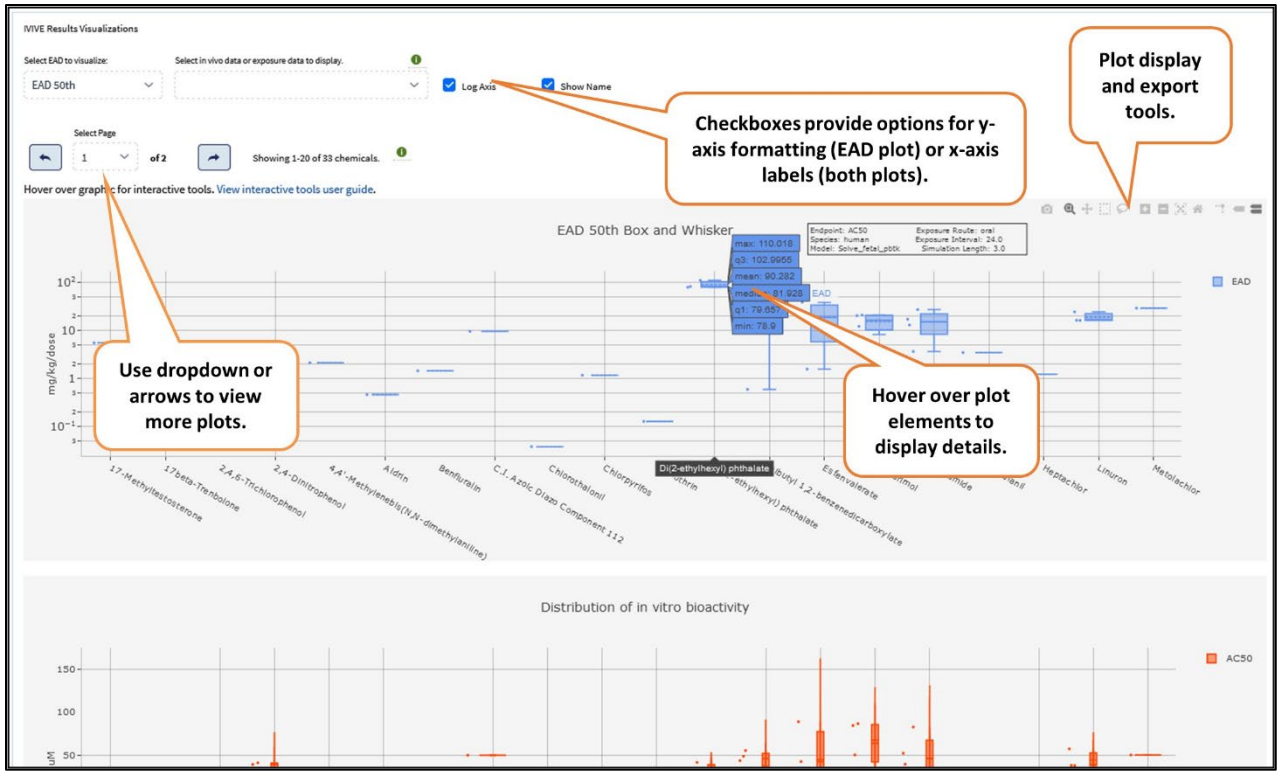


Figure 13. Options for customizing the plot display and interacting with the plot data. [Screenshot from ICE 4.0, updated 14 Apr 2023.]

Using Results to Query Other ICE Tools

Use the "Send filtered results to" dropdown list **(Figure 14)** next to the download icons to send result parameters (selected chemicals and cHTS assays and Modes of Action) to other ICE tools. For details on the use and outputs of these tools, refer to their user guides.

- Click "Search" to query and retrieve all data in ICE for the selected chemicals.
- Click "Chemical Quest" to query ICE for chemicals that are structurally similar to your chemicals.
- Click "Curve Surfer" to display activity curves for chemicals that have cHTS assay data in ICE.
- Click "PBPK" to send chemicals to the PBPK tool. This tool generates predictions of tissue-specific chemical concentration profiles following a dosing event.
- Click "Chem Characterization" to view physicochemical and ADME properties and consumer use categories for these chemicals.

You can also copy the chemical names, CASRNs, DTXSIDs, SMILES strings, or quantitative structure–activity relationship (QSAR)-ready SMILES strings to the clipboard.

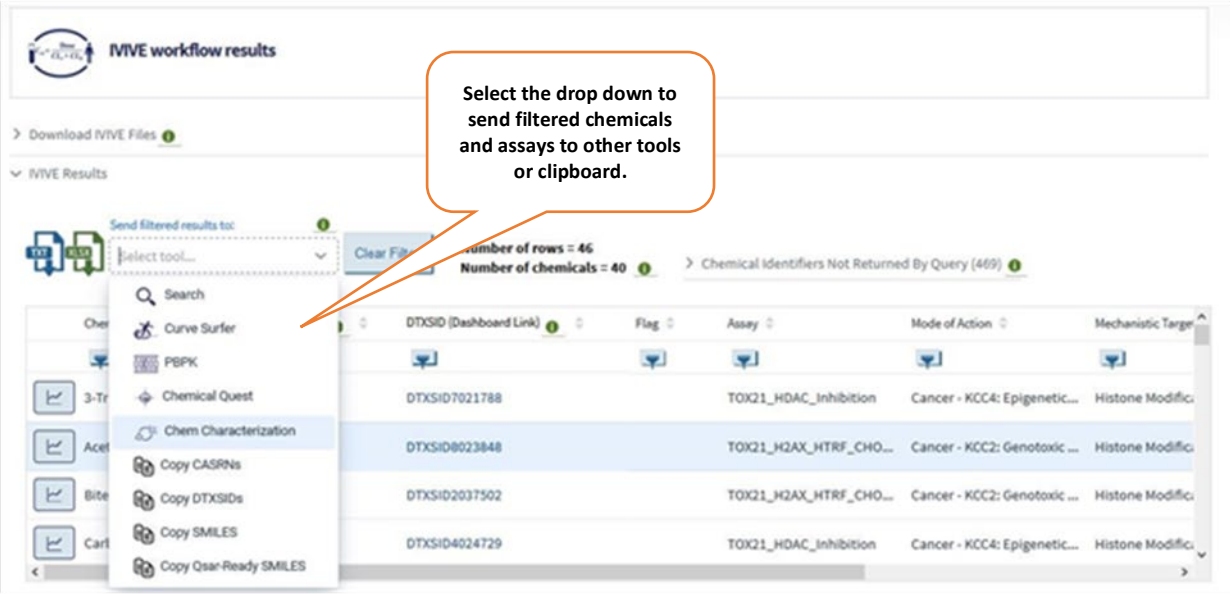


Figure 14. Send filtered results to other ICE tools. [Screenshot from ICE 4.0, updated 31 Mar 2023.]

Appendix 1: How IVIVE is Calculated

Chemical-specific PK Parameters

To predict EAD, the IVIVE tool requires chemical-specific PK parameters, as well as a measure of in vitro assay activity (specified as AC50 or ACC as described above for cHTS assays or activity concentration supplied by the user).

Parameters required for all models available in the ICE IVIVE tool (**Figure 15**) include:

- Fraction of chemical unbound to plasma protein (fu).
- Intrinsic clearance from liver (CLint).
- Renal clearance.
- Molecular weight (MW).

Additional PK parameters required for executing the Solve_3comp, Solve_pbtk, Solve_gas_pbtk, and Solve_fetal_pbtk models from the EPA's httk package include:

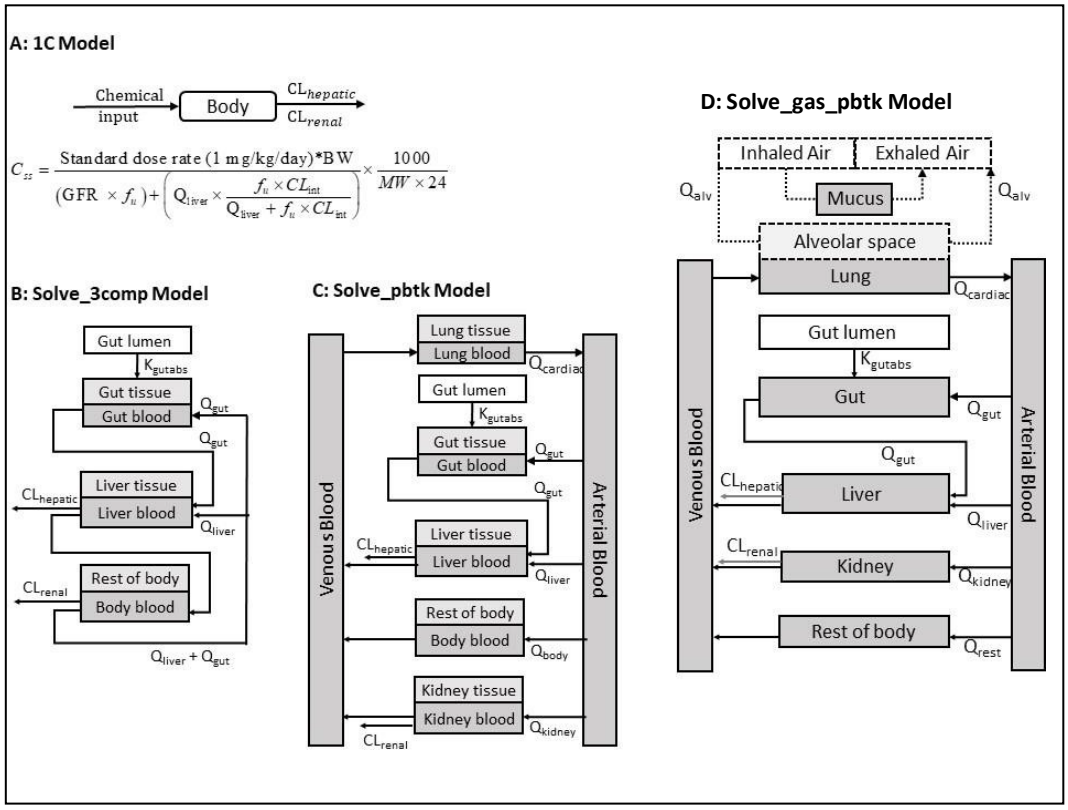
- Octanol-water partition coefficient (logP).
- Henry's Law constant (a measure of solubility of gas in a liquid).
- Acid-dissociation constant (pKa).
- Tissue:plasma partition coefficients (liver, kidney, lung, and rest-of-body).

ICE currently provides predicted values for most of these parameters for over 800,000 chemicals through OPERA (Mansouri et al. 2018). OPERA is a free and open-source/open-data suite of QSAR

models providing predictions of fu, CLint, and physicochemical properties (i.e., logP, Henry's Law constant, pKa, etc.). The tissue:plasma partition coefficients required by the httk package models are calculated using the "predict_partitioning_schmitt" function in the httk R package (Schmitt et al. 2008; Pearce et al. 2017).

PK Models Used in the ICE IVIVE Tool

The ICE IVIVE tool first uses PK models to estimate a plasma concentration of 1 mg/kg/dose. It then applies linear extrapolation to estimate the EAD that would result in a plasma concentration equivalent to the activity concentration of the selected assays. **Figure 15** shows the structure of each PK model used in the IVIVE tool.



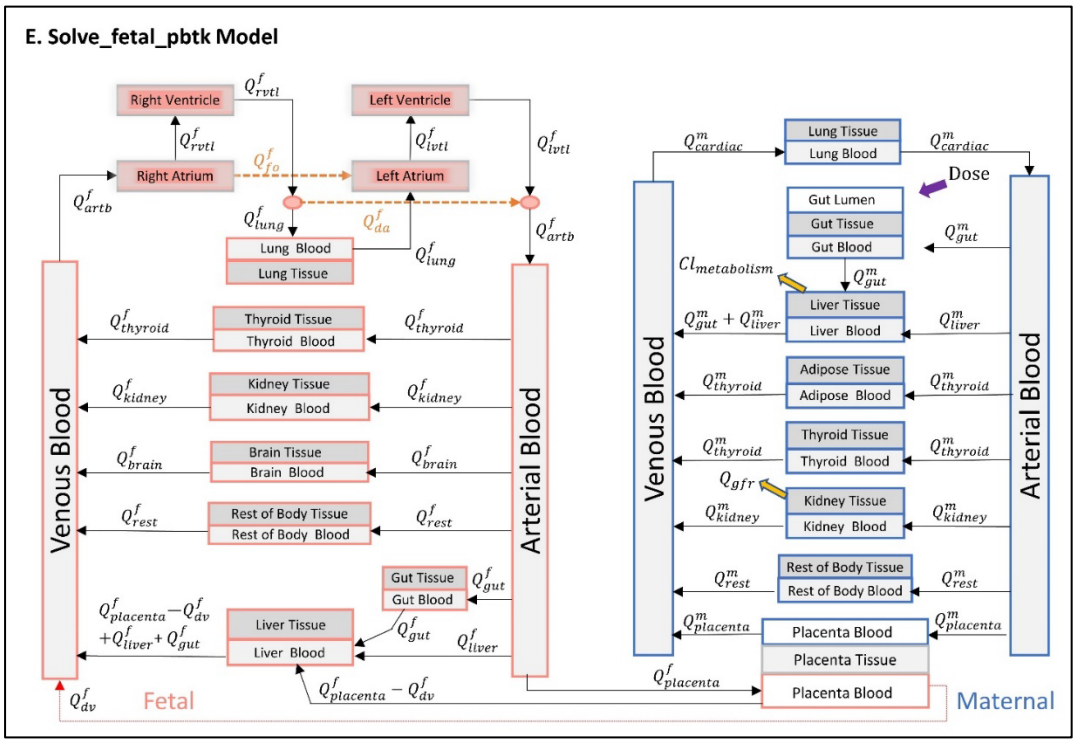


Figure 15. The structure of PK models used in the IVIVE tool. For the Solve_fetal_pbtk model (E), abbreviations beginning with Q^m indicate blood flow in maternal tissues, and abbreviations beginning with Q^f indicate blood flow in fetal tissues. GFR, glomerular filtration rate; K_{gutabs}, rate constant of gut absorption (h^{-1}).

1C Model

The 1C PK model (**Figure 15A**) is a one-compartment model that assumes 100% absorption to estimate Css at a dose of 1 mg/kg/day. This model uses a Monte Carlo simulation that accounts for inter-individual physiological variations (Wetmore et al. 2012) to estimate variability across individuals. Elimination of chemicals is assumed to be by hepatic metabolism and passive glomerular filtration.

Css is calculated using the following equation:

$$Css = 1 / [(GFR \times fu) + (Q_{liver} \times fu \times CLint) / (Q_{liver} + fu \times CLint)] \times (BW / 24) \times (1000 / MW)$$

In this equation:

- BW: body weight (Kg)
- CLint: intrinsic clearance from liver (L/h)
- GFR: glomerular filtration rate (L/h)
- MW: molecular weight (g/mol)
- Q_{liver}: liver blood flow rate (L/h)

Css is used as input for a reverse dosimetry calculation that produces an EAD corresponding to the in vitro activity concentration (Wetmore et al. 2012; Casey et al. 2018).

The EAD values provided in the interactive results table and plots corresponding to unbound chemical concentration at steady state, which is calculated as follows:

EADadj = ACC (or AC50) x 1 / (Css x fu) (mg/kg/day)

Solve_3comp Model

Solve_3comp (**Figure 15B**) uses the Solve_3comp function from the httk R package (httk v 2.2.2) (Pearce et al. 2017). The function uses a three-compartment PK model in the httk package to solve for plasma and tissue compartment concentrations as functions of time. The three compartments included are gut, liver, and rest-of-body, each of which is described as perfusion rate-limited. The three-compartment model also assumes an arbitrary "fast" absorption rate constant of 1 h^{-1} from the gut lumen into the gut tissue. Elimination of chemicals is assumed to be by hepatic metabolism and passive glomerular filtration. In the ICE IVIVE tool, the Solve_3comp function is used to estimate plasma Cmax following an injection or oral dose of 1 mg/kg/dose at user-select dosing interval. For a single daily dosage, the default dosing interval is 24 hours.

Solve_pbtk Model

Solve_pbtk (**Figure 15C**) uses the Solve_pbtk function from the httk R package (httk v 2.2.2) (Pearce et al. 2017). The function uses a multiple-compartment PBPK model in the httk package to solve for plasma and tissue compartment concentrations as functions of time. The model can be run for human or rat. The model includes gut, artery, vein, lung, liver, kidney, and rest-of-body compartments. Each tissue compartment is regarded as perfusion rate-limited. The rate of change of the amount of chemical in each tissue compartment is described by mass balance differential equations. The elimination of chemicals is assumed to be by hepatic metabolism and passive glomerular filtration. In the ICE IVIVE tool, the Solve_pbtk function is used to estimate plasma Cmax following an IV injection or oral dose of 1 mg/kg/dose at a user-selected dosing interval. For a single daily dosage, the default dosing interval is 24 hours.

Solve_gas_pbtk Model

Solve_gas_pbtk (**Figure 15D**) uses the Solve_gas_pbtk function from the httk R package (httk v 2.2.2) (Pearce et al. 2017, Linakis et al. 2020). The function uses an inhalation route-specific PBPK model in the httk package to solve for plasma and tissue compartment concentrations as functions of time. The model can be run for human or rat subjects. The model contains the same compartments as the Solve_pbtk model but has an additional dosing module for inhalation (gas) route of exposure. Each tissue compartment is regarded as perfusion rate-limited. The rate of change of the amount of chemical in each tissue compartment is described by mass balance differential equations. The elimination of chemicals is assumed to be by hepatic metabolism and passive glomerular filtration. The model currently only simulates exposure through air concentration, which is given over the exposure length. In the ICE IVIVE tool, the Solve_gas_pbtk function is used to estimate plasma Cmax following a gas exposure of 1 ppmv or uM at a user-selected dosing length and interval.

Solve_fetal_pbtk Model

Solve_fetal_pbtk (**Figure 15E**) uses the Solve_fetal_pbtk function from the httk R package (httk v 2.2.2) (Pearce et al. 2017, Kapraun et al. 2022). The function uses a PBPK model to describe chemical distribution for exposure in humans via the oral or IV injection route starting at a specified day of

gestation within the range of day 91 to day 280. The function solves for maternal and fetal compartment concentrations as functions of time. The model includes both maternal and fetal compartments and a placenta modeled as a joint organ shared by mother and fetus. The maternal compartments include gut, artery, vein, lung, liver, adipose, thyroid, kidney, placenta, and rest-of-body compartments. The fetal compartments include gut, artery, vein, right ventricle, left ventricle, right atrium, left atrium, lung, liver, brain, thyroid, kidney, and rest-of-body. As for the other models, each tissue compartment is regarded as perfusion rate-limited, and the rate of change of the amount of chemical in each tissue compartment is described by mass balance differential equations. The elimination of chemicals is assumed to be by hepatic metabolism and passive glomerular filtration. In the ICE IVIVE tool, the Solve_fetal_pbtk function is used to estimate maternal or fetal plasma Cmax following an IV injection or oral dose of 1 mg/kg/dose at a user-selected dosing interval.

For all the httk models, plasma Cmax is used as an input for a reverse dosimetry calculation. Assuming a linear relationship between external dose and internal plasma concentration, EAD is calculated as follows:

EAD = ACC (or AC50) x 1 / Cmax (mg/kg/day)

Appendix 2: References and Other Resources

References Cited in this Document

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Ring CL, Arnot JA, Bennett DH, Egeghy PP, Fantke P, Huang L, et al. 2019. Consensus modeling of median chemical intake for the U.S. population based on predictions of exposure pathways. Environ Sci Technol 53(2):719-732. doi: 10.1021/acs.est.8b04056.

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Wetmore BA, Wambaugh JF, Ferguson SS, Sochaski MA, Rotroff DM, Freeman K, et al. 2012.Integration of dosimetry, exposure, and high-throughput screening data in chemical toxicity assessment. Toxicol Sci 125(1): 157–174. doi:10.1093/toxsci/kfr254

For More Information

https://cran.r-project.org/web/packages/httk/index.html: Functions and data tables for simulation and statistical analysis of chemical toxicokinetics ("TK") as in Pearce et al. (2017).

https://github.com/NIEHS/ICE_IVIVEpipeline: R Notebook for the downloadable IVIVE standalone application.

https://ice.ntp.niehs.nih.gov/DATASETDESCRIPTION: Descriptions of data sets included in ICE, including the cHTS data set.

Appendix 3: Abbreviations

This list includes both abbreviations used within this User Guide and abbreviations used in the ICE IVIVE tool interface.

AC50: concentration that causes a half-maximal response

ACC: activity concentration at cutoff

ADME: absorption, distribution, metabolism, and excretion

BW: body weight

CASRN: Chemical Abstracts Service Registry Number

CEBS: Chemical Effects in Biological Systems (National Toxicology Program database)

cHTS: curated high-throughput screening

CLhepatic: clearance rate from liver (Figure 15)

CLint: intrinsic clearance from liver

CLrenal: renal clearance rate (Figure 15)

Cmax: maximum concentration

Css: steady-state concentration

DTXSID: Distributed Structure-Searchable Toxicity (DSSTox) Substance Identifier (U.S. Environmental Protection Agency)

EAD: equivalent administered dose

EADadj: EAD corresponding to unbound chemical concentration in plasma

EADtotal: EAD corresponding to total chemical concentration in plasma

EPA: U.S. Environmental Protection Agency

fu: fraction of chemical unbound to plasma protein

GFR: glomerular filtration rate

httk: high-throughput toxicokinetics

InChIKeys: hashed International Chemicals Identifiers

IV: intravenous

IVIVE: in vitro to in vivo extrapolation

LD50: in traditional animal tests for acute systemic oral or dermal toxicity, the dose that causes death in 50 percent of the animals tested

logP: octanol-water partition coefficient

MW: molecular weight

OPERA: Open Structure–activity/property Relationship App

PBPK: physiologically based pharmacokinetic

PK: pharmacokinetics

pKa: acid-dissociation constant

Qi: blood flow to tissue i (Figure 15)

QSAR: quantitative structure–activity relationship

SEEM3: Systematic Empirical Evaluation of Models

SMILES: Simplified molecular-input line-entry system