

IVIVE Simulation Examples

Kristen Windoloski

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If you have not already read the *Introduction to the ToCS App* vignette, it is highly recommended to do so to get a general idea of the app's layout and obtain a detailed description of common user inputs across all output modules. Users should also review the README file on the ToCS GitHub page (github.com/KristenWindoloski/ToCS) to setup ToCS if they have not accessed the app yet. This vignette assumes that you have access the ToCS app GUI already.

Introduction

This vignette provides three examples that use the ToCS app to generate oral equivalent doses (OEDs) by in vitro in vivo extrapolation (IVIVE), each example with different parameters selected. Two of the three examples also incorporate chemical exposure data into the program to generate bioactivity exposure ratios for chemical prioritization. To begin, open the app by using any of the methods described in the README file. You have correctly accessed the app if your screen looks like the image below.

The screenshot displays the opening interface of the Toxicokinetic Chemical Simulator (ToCS) app. The interface features a top navigation bar with the following tabs: "Toxicokinetic Chemical Simulator (ToCS)", "General Parameters", "Model Specifications", "Compound Selection", "Advanced (Optional) Parameters", and "Run Simulation". The "General Parameters" tab is currently selected and highlighted.

The main content area is divided into three vertical panels:

- INSTRUCTIONS:** Contains text explaining the app's purpose and providing links for additional guidance. It states: "Fill out the prompts on each of the above tabs moving left to right. Then, click the 'Run Simulation' tab to run the simulation or reset all selections." It also lists the four toxicokinetic (TK) outputs: 1) Concentration-time profiles, 2) Steady state (SS) concentrations, 3) In vitro in vivo extrapolation (IVIVE), and 4) Parameter calculations. It mentions the app uses the U.S. EPA's R package 'httk' and provides links to the vignettes and GitHub repository.
- OUTPUT:** Features a dropdown menu labeled "Select the desired output." with a "Select" option. Below the dropdown, a red error message states: "Must not be equal to Select."
- SPECIES:** Features a dropdown menu labeled "Select the species to analyze." with a "Select" option. Below the dropdown, a red error message states: "Must not be equal to Select." There is also a section asking "Do you want to use human in vitro data if in vitro data for the selected species is missing?" with another "Select" dropdown and a red error message: "Must not be equal to Select."

The opening interface to the ToCS app.

Example 1

Let's say we want to perform IVIVE for eight chemicals that we have bioactivity data for. Since the IVIVE produces an OED for each bioactive concentration, assume that we want to transform the nominal bioactivity data to a free concentration in vitro. When the solution is outputted, we want the 5th dose quantile human plasma OED calculated from the pbtk model. Assume that we also have chemical exposure estimates for all eight chemicals and wish to plot these against the OEDs and generate bioactivity exposure ratios.

General Parameters Tab

Since we want to perform IVIVE, we select the *In vitro in vivo extrapolation (IVIVE)* option under the drop down menu from the *Output* card. Then under the *Species* card, we select *Human* for the first drop down and *Yes* for the second drop down. As with other vignettes, we could have selected *No* for the second drop down menu under the *Species* card and it would not make a difference in the simulation results since the selected species is already human. Thus, the completed *General Parameters* tab should look like the image below.

The screenshot shows the 'General Parameters' tab of the Toxicokinetic Chemical Simulator (ToCS). The interface is divided into three main sections: INSTRUCTIONS, OUTPUT, and SPECIES.

INSTRUCTIONS: This section provides guidance on using the simulator. It states: 'Fill out the prompts on each of the above tabs moving left to right. Then, click the 'Run Simulation' tab to run the simulation or reset all selections.' It also lists the four toxicokinetic (TK) outputs: 1) Concentration-time profiles, 2) Steady state (SS) concentrations, 3) In vitro in vivo extrapolation (IVIVE), and 4) Parameter calculations. It mentions that the application uses the U.S. EPA's R package 'httk' and provides links for more information. Finally, it offers additional guidance and a link to report issues or suggestions for improvement.

OUTPUT: This section allows the user to select the desired output. A dropdown menu is set to 'In vitro in vivo extrapolation (IVIVE)'.

SPECIES: This section allows the user to select the species to analyze. A dropdown menu is set to 'Human'. Below this, there is a question: 'Do you want to use human in vitro data if in vitro data for the selected species is missing?'. A dropdown menu for this question is set to 'Yes'.

The completed General Parameters tab for example 1.

Model Specifications Tab

Under the *Dosing* card, we see that there are no user specifications to be made for this module. However, there are several user choices to be made under the *Model* card. Since we want to use the pbtk model for IVIVE, we select *pbtk* for the first drop down. For the second drop down we select *No* and decide to not make compounds with only in silico generated parameters available for this example. For the third item under the *Model* card, we have to upload a csv file with the bioactive concentration (uM) of each of the compounds we

want to simulate. Therefore, we upload the following csv table. Note that the table must have the following exact format.

A csv file with bioactivity data for all eight chemicals. For the purpose of this example, the bioactivity data provided in this csv is fictional.

ChemicalName	CAS	BioactiveConcentration
Acetamiprid	135410-20-7	1.20
2,4-db	94-82-6	50.30
Acephate	30560-19-1	22.90
Acetochlor	34256-82-1	0.95
Alachlor	15972-60-8	3.24
Aldicarb	116-06-3	5.90
Ametryn	834-12-8	13.60
Amitraz	33089-61-1	8.79

Then, the fourth drop down menu asks the user whether they want to return a single OED per compound (a selected quantile) or all OED samples per compound. Since we only want the 5th OED quantile (95th steady state concentration quantile), we select *Only return a specified dose quantile (default)*. This prompts the appearance of an additional numeric input box where we want to enter the desired steady state concentration quantile. Since we want the 95th steady state concentration quantile (5th OED quantile), we leave the input as *0.95*. Thus, the completed *Model Specifications* tab should look like the two images below.

Toxicokinetic Chemical Simulator (ToCS) General Parameters **Model Specifications** Compound Selection Advanced (Optional) Parameters Run Simulation

DOSING

No options for this category.

MODEL

Select the model to simulate. If a species other than 'Human' is selected, '3compartments' must be chosen.

pbtk

Select whether to use in silico generated parameters for compounds with missing in vitro data. These parameters will not overwrite existing in vitro data, and it will expand the number of compounds available.

No, do not load in silico parameters

Upload one CSV file with your species' in vitro bioactive concentrations (in uM units) for all compounds. The CSV should have three column names in the following order with one row per chemical: ChemicalName, CAS, BioactiveConcentration.

Browse... SampleBioData_8Che

Upload complete

Select whether to return all oral equivalent dose (OED) samples for each compound or a selected quantile.

Only return a specified dose quantile

The completed model specifications tab for example 1 showing the upper part of the model card.

Toxicokinetic Chemical Simulator (ToCS) General Parameters **Model Specifications** Compound Selection Advanced (Optional) Parameters Run Simulation

DOSING

No options for this category.

MODEL

parameters will not overwrite existing in vitro data, and it will expand the number of compounds available.

No, do not load in silico parameters

Upload one CSV file with your species' in vitro bioactive concentrations (in uM units) for all compounds. The CSV should have three column names in the following order with one row per chemical: ChemicalName, CAS, BioactiveConcentration.

Browse... SampleBioData_8Che

Upload complete

Select whether to return all oral equivalent dose (OED) samples for each compound or a selected quantile.

Only return a specified dose quantile (default)

Enter the steady state concentration quantile (as a decimal) to be used in the OED calculation. Selecting the 95th concentration quantile will output the 5th OED quantile.

0.95

The completed model specifications tab for example 1 showing the lower part of the model card.

Compound Selection Tab

Different from the other modules and vignettes, the first drop down menu under the *Preloaded Compounds* card has the user select a set of assumptions to implement regarding in vitro and in vivo bioactivity as well as

metabolic clearance. The user can select from the following options:

- NULL:
 - Default assumptions applied (listed below) or customizable in the Advanced Parameters tab
 - Restrictive metabolic clearance (protein binding taken into account in liver clearance)
 - Treats the total specified concentration or tissue as bioactive in vivo
 - Treats the nominal concentration in vitro as bioactive
- Honda1:
 - Restrictive metabolic clearance (protein binding taken into account in liver clearance)
 - Treats the unbound (free) venous plasma concentration in vivo as bioactive
 - Treats the unbound (free) concentration in vitro as bioactive
- Honda2:
 - Restrictive metabolic clearance (protein binding taken into account in liver clearance)
 - Treats the unbound (free) venous plasma concentration in vivo as bioactive
 - Treats the nominal concentration in vitro as bioactive
- Honda3:
 - Restrictive metabolic clearance (protein binding taken into account in liver clearance)
 - Treats the total venous plasma concentration in vivo as bioactive
 - Treats the nominal concentration in vitro as bioactive
- Honda4:
 - Non-restrictive metabolic clearance (protein binding not taken into account in liver clearance)
 - Treats the total specified tissue concentration in vivo as bioactive
 - Treats the nominal concentration in vitro as bioactive

For more details, see the EPA's htk documentation and/or the following publication

<https://doi.org/10.1371/journal.pone.0217564>. Since we want to use the unbound (free) concentration in vitro as bioactive instead of the nominal concentration, we select the *Honda1* assumption for the first drop down menu. This then results in a new box below asking the user to specify the fraction fetal bovine serum. We apply the assumption that it is 0.1 for the bioactivity assays for our selected compounds and leave the box at 0.1. Another box below also appears and contains a list of preloaded compounds that we can select from. We search for the same eight compounds that we uploaded bioactivity data for and see that they are all present in the preloaded list. Thus, we select those compounds and ignore the *Uploaded Compounds* card. The completed *Compound Selection* tab should look like the images below.

Toxicokinetic Chemical Simulator (ToCS)
General Parameters
Model Specifications
Compound Selection
Advanced (Optional) Parameters
Run Simulation

INSTRUCTIONS

Below is a downloadable folder containing information on upload file formatting if compounds to be analyzed are not included in the preloaded compounds list. The uploaded file must be formatted exactly how the 'SampleCSV' file is structured (keeping columns even if there is no data). The header descriptions of the CSV and the minimal data inputs required for the CSV are in the 'DataDescriptions' and 'RequiredData' PDFs, respectively.

[Uploaded Compound File Folder](#)

PRELOADED COMPOUNDS

bioactive concentration in vitro, the Honda1 assumption is recommended. Leave on 'NULL' if no assumptions are to be applied. See the 'IVIVE Simulation Examples' vignette for the description of the below assumption categories.

Honda1

Enter the volume fraction of fetal bovine serum used in the in vitro assay.

0.1

Select any preloaded compounds. Search through the list by clicking on the box and scrolling or typing in a name. The list may not show all available compounds. Click on a compound to select it. You may select multiple.

94-82-6, 2,4-db

30560-19-1, Acephate

135410-20-7, Acetamiprid

34256-82-1, Acetochlor

15972-60-8, Alachlor

116-06-3, Aldicarb

834-12-8, Ametryn

33089-61-1, Amitraz

UPLOADED COMPOUNDS

Upload a CSV file of data for compounds not in the preloaded list (if desired). See the 'Instructions' panel to the left for directions on file formatting requirements.

Browse...
No file selected

The completed compound selection tab for example 1.

Advanced (Optional) Parameters Tab

In this tab, we have the opportunity to upload chemical exposure data (as a csv file) into the program in order to visualize these exposure estimates against the model-outputted OEDs as well as a generate bioactivity exposure ratios, which can guide chemical prioritization for potential risk. Suppose we have the following exposure data file:

A csv file with exposure data (mg/kg BW/day) for all eight chemicals. These exposure estimates were gathered from the EPA CompTox Dashboard. NHANES estimates were used if available. Otherwise, the EPA's SEEM3 model predictions were used.

ChemicalName	CAS	Upper	Median	Lower
Acetamiprid	135410-20-7	2.39e-04	5.99e-07	NA
2,4-db	94-82-6	1.29e-04	1.31e-06	NA
Acephate	30560-19-1	1.40e-06	3.37e-07	4.79e-08
Acetochlor	34256-82-1	2.00e-07	4.93e-08	4.74e-09
Alachlor	15972-60-8	1.40e-06	2.7e-07	NA
Aldicarb	116-06-3	2.39e-03	3.03e-06	NA
Ametryn	834-12-8	1.43e-03	2.23e-06	NA

ChemicalName	CAS	Upper	Median	Lower
Amitraz	33089-61-1	9.77e-04	1.24e-06	NA

This file can be uploaded under the *Output Specification* card on the right. For help with exposure file formatting, please refer to the *Introduction to ToCS* vignette and/or the *Exposure Data File Folder* download option below the uploader in the interface. We will leave the other options on this tab at their default values and then proceed to the final *Run Simulation* tab.

Toxicokinetic Chemical Simulator (ToCS)

General Parameters Model Specifications Compound Selection **Advanced (Optional) Parameters** Run Simulation

MODEL CONDITIONS

Enter the number of Monte Carlo samples generated for each compound.

Enter the minimum acceptable chemical fraction unbound in presence of plasma proteins. All values below this will be set to this value.

Select whether to adjust the chemical fraction unbound in presence of plasma proteins for lipid binding.

Yes, adjust the fraction of unbound plasma (default)

Select whether to use regressions when calculating partition coefficients.

Use regressions (default)

MODEL SOLVER

No options for this category.

BIOAVAILABILITY

Enter a default value for the Caco-2 apical-to-basal membrane permeability (denoted Caco2.Pab, 10^{-6} cm/s).

Select whether to use the Caco2.Pab value set above to estimate F_{abs} (the in vivo measured fraction of an oral dose absorbed from the gut lumen into the gut) if bioavailability data is unavailable.

Use the Caco2.Pab value selected above (default)

Select whether to use the Caco2.Pab value set above to calculate F_{gut} (the in vivo measured fraction of an oral dose that passes gut metabolism and clearance) if bioavailability data is unavailable.

Use the Caco2.Pab value selected above (default)

Select whether to overwrite in vivo F_{abs} and F_{gut} data (if available).

Do not overwrite in vivo values

OUTPUT SPECIFICATION

Upload a CSV file of exposure data for the selected compounds. See the downloadable folder below for file upload formatting details.

Browse...

SampleExpData_8Che

Upload complete

[Exposure Data File Folder](#)

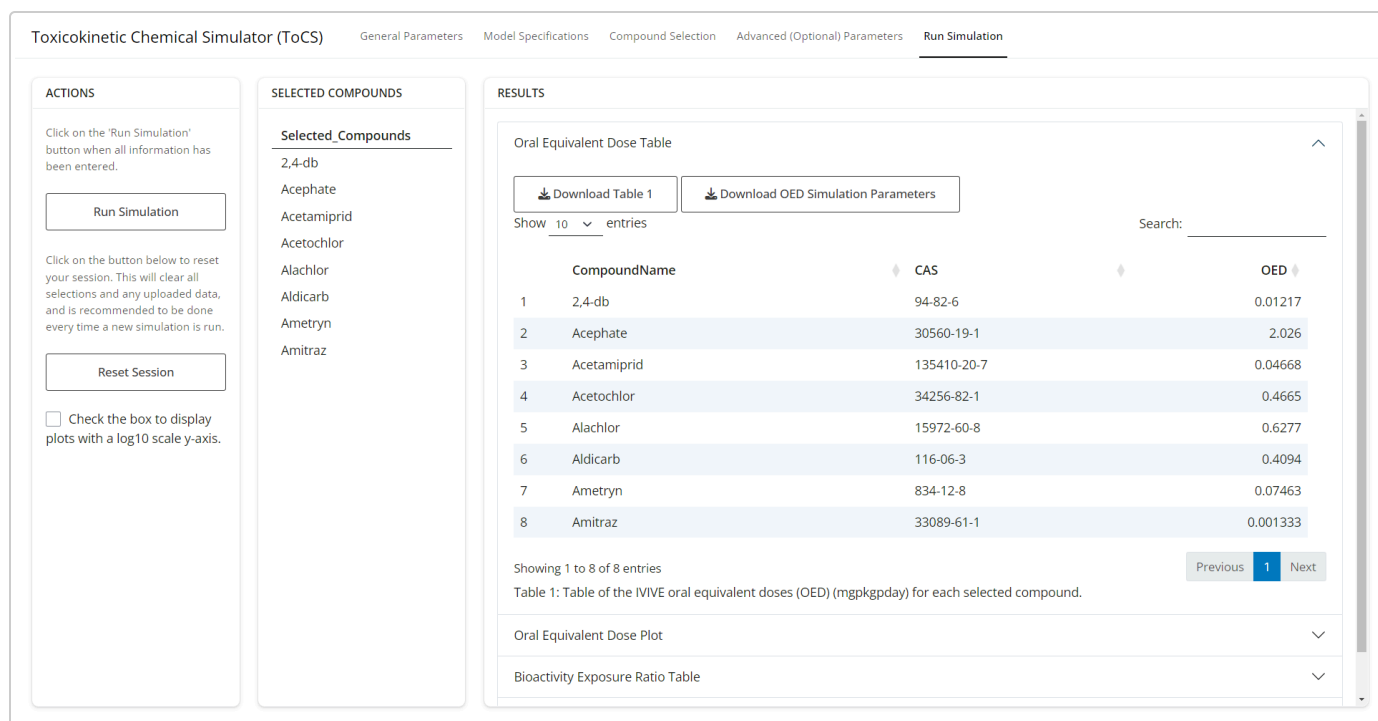
Select the dose output units from either mg/kg BW/day (mg/kgpday) (default) or umol/kg BW/day (umol/kgpday).

mg/kgpday

The completed advanced parameters tab for example 1.

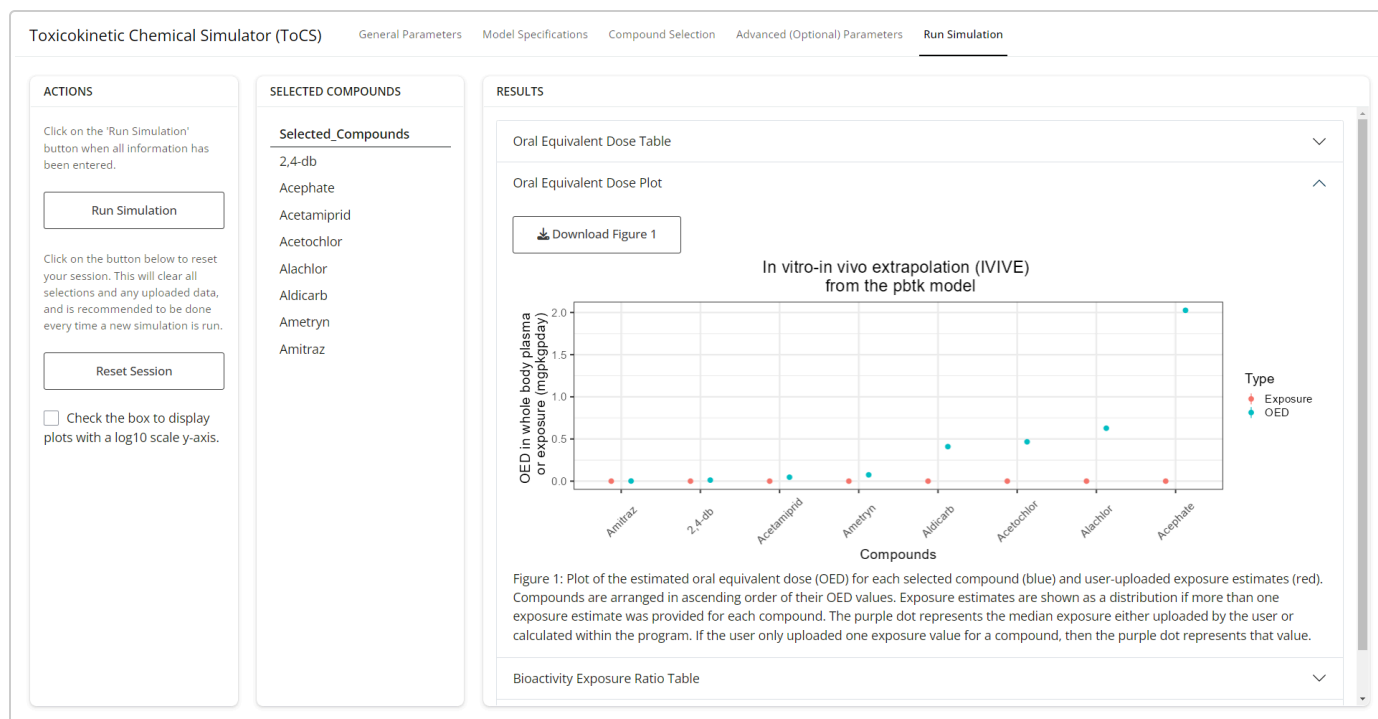
Run Simulation Tab

Now that all user parameter selections have been made and all compounds appear under the *Select Compounds* card, we click the *Run Simulation* button. Once the simulation is complete, the user's interface should look like the image below. The first tab shows a table of the OED for each compound. The table is available for download by the user if the user clicks the *Download Table 1* button. The user is also able to download all of the simulation parameters and chemical information used in the simulation by clicking the *Download OED Simulation Parameters* tab.



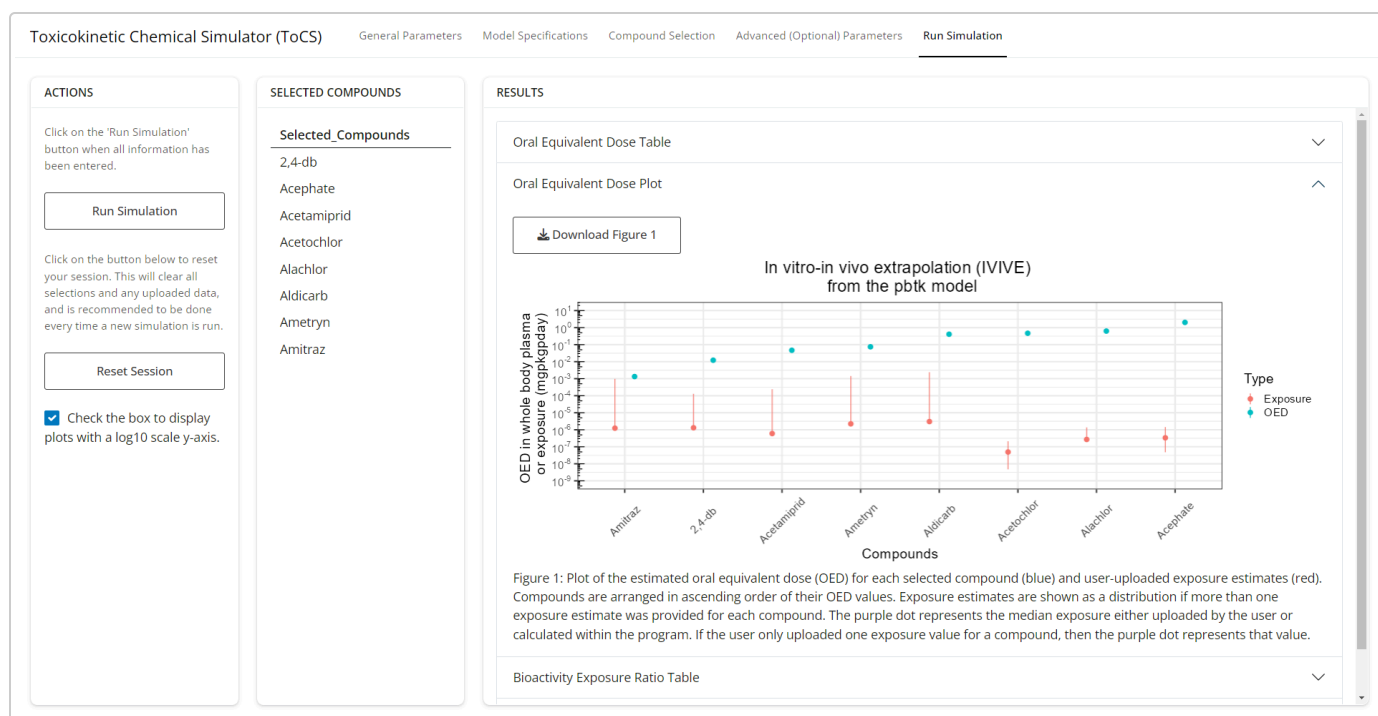
The completed run simulation tab for example 1 showing the expanded OED table tab.

The image below is the completed OED plot tab, which shows the OED (in blue) for the selected quantile for each compound plotted in ascending order. The plot also shows the exposure data estimates (in pink) from the uploaded exposure data file on the previous page next to each respective chemical so users can compare the oral equivalent dose needed for bioactivity and the exposure estimate. The plot shown below uses a linear y-axis, but as we can see, this makes it challenging to visually see the difference between the smaller OEDs as well as the distribution for the exposure estimates.



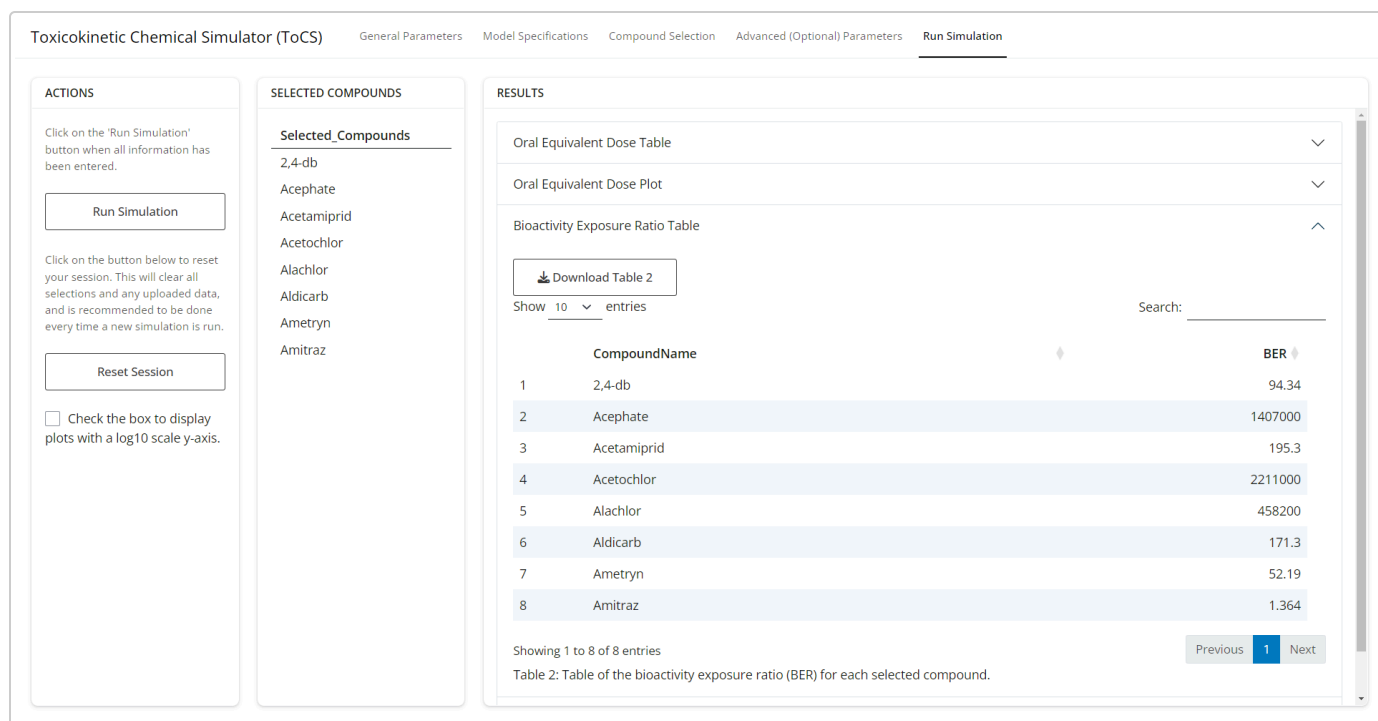
The completed run simulation tab for example 1 showing the expanded OED plot tab where the plot has a linear scale y-axis.

Therefore, we can click the box under the *Actions* card to change the scale of the y-axis to a log10 scale. That then results in the image shown below, which allows the user to view the differences in magnitudes of all OEDs and exposure estimates. Two chemicals have three exposure estimates available (lower, median, and upper), which is clearly seen by the median pink dot with bars extending in both directions, while the remaining chemicals only have two exposure estimates uploaded (median and upper). Those chemicals show a pink dot which represents the median exposure and one bar which reaches the upper exposure limit. The user can download either the linear or log10 scale plot by clicking the *Download Figure 1* button.



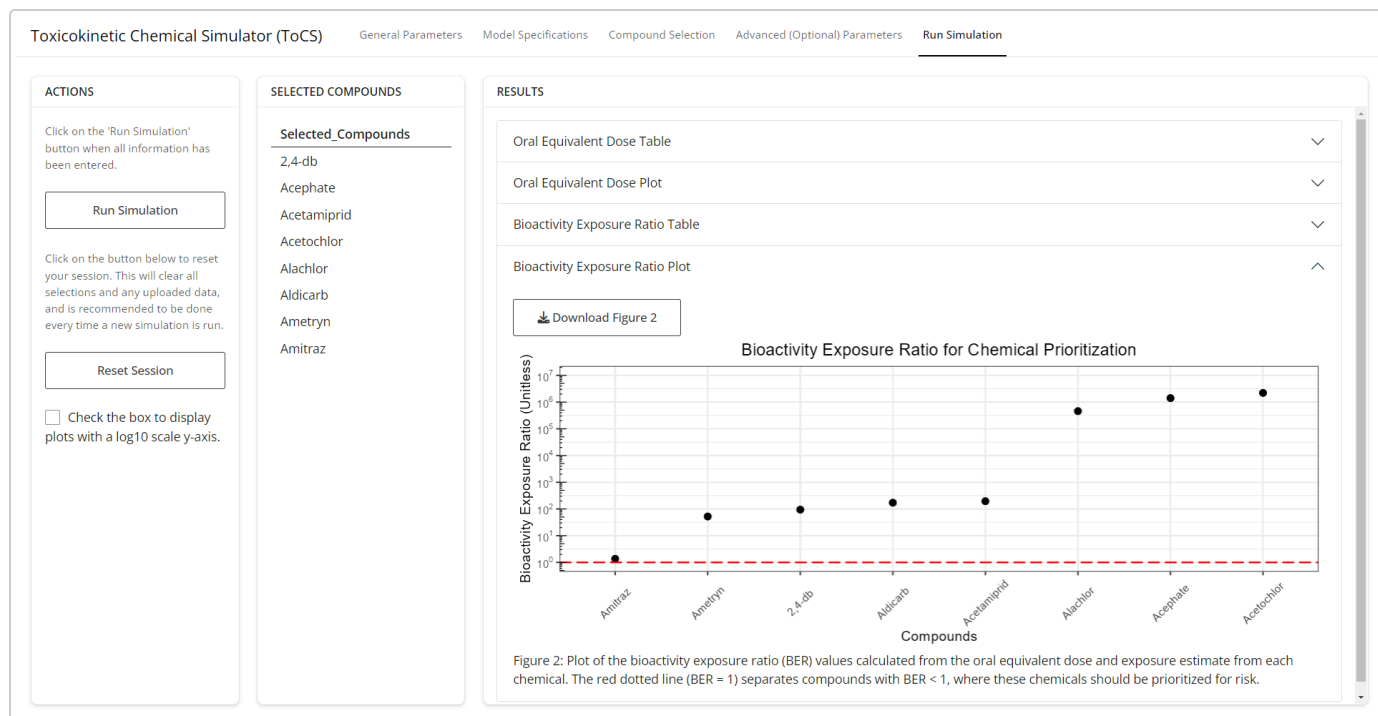
The completed run simulation tab for example 1 showing the expanded OED plot tab where the plot has a log10 scale y-axis.

The image below shows the bioactivity exposure ratio (BER) table output for the calculated OEDs and uploaded exposure estimates. This is computed as the OED divided by the upper exposure estimate. As with Table 1, users can download this table by clicking the "Download Table 2" button.



The completed run simulation tab for example 1 showing the expanded bioactivity exposure ratio (BER) table tab.

The final output for the IVIVE module is given below and shows a plot of the BER for each chemical. The red dotted line on the plot indicates the threshold for chemical prioritization (BER = 1), where any chemicals that fall below that threshold should be prioritized for risk. In this simulation though, all BERs are greater than one. As with the previous plot, the user has the opportunity to download this plot by clicking the “Download Figure 2” button above the plot.



The completed run simulation tab for example 1 showing the expanded BER plot tab where the plot has a log10 scale y-axis.

If we wanted to run another simulation, we would click the *Reset Session* button under the *Actions* button, which would clear all parameter inputs and simulations and return the interface to the *General Parameters* tab.

Example 2

Let's say we want to perform human IVIVE for ten chemicals that we have bioactivity data for. Assume that we want to use the nominal plasma in vitro bioactivity data as the bioactive concentration instead of the free concentration in vitro, and we want to use restrictive clearance. When the solution is outputted, suppose that we want to view all generated plasma OED samples calculated from the 3compartmentss model. Also suppose that we have chemical exposure estimates for all ten chemicals and would like to use them for this analysis.

General Parameters Tab

Since we want to perform IVIVE, we select the *In vitro in vivo extrapolation (IVIVE)* option under the drop down menu from the *Output* card. Then under the *Species* card, we select *Human* for the first drop down and *Yes* for the second drop down. As with other vignettes, we could have selected *No* for the second drop down menu under the *Species* card and it would not make a difference since the selected species is already human. Thus, the completed *General Parameters* tab should look like the image below.

Toxicokinetic Chemical Simulator (ToCS)

General Parameters

Model Specifications

Compound Selection

Advanced (Optional) Parameters

Run Simulation

INSTRUCTIONS

Fill out the prompts on each of the above tabs moving left to right. Then, click the 'Run Simulation' tab to run the simulation or reset all selections.

ToCS provides four toxicokinetic (TK) outputs: 1) Concentration-time profiles, which returns chemical concentrations in bodily compartments over time, 2) Steady state (SS) concentrations, which returns SS concentrations in bodily compartments from an oral infusion, 3) In vitro in vivo extrapolation (IVIVE), which returns oral equivalent doses to in vitro bioactive concentrations, 4) Parameter calculations, which returns elimination rates, volumes of distribution, tissue to unbound plasma partition coefficients, half-lives, and total plasma clearances.

This application uses the U.S. EPA's R package 'httk'. For more information on 'httk', refer to <https://doi.org/10.18637/jss.v079.i04> and/or <https://cran.r-project.org/web/packages/httk>

For additional guidance on ToCS, please refer to the vignettes (<https://github.com/KristenWindolowski/ToCS/tree/main/vignettes>). To report issues or suggestions for improvement, visit <https://github.com/KristenWindolowski/ToCS/issues>.

OUTPUT

Select the desired output.

In vitro in vivo extrapolation (IVIVE) ▾

SPECIES

Select the species to analyze.

Human ▾

Do you want to use human in vitro data if in vitro data for the selected species is missing?

Yes ▾

The completed General Parameters tab for example 2.

Model Specifications Tab

Under the *Dosing* card, there are no options to select from. Under the *Model* card, we select *3compartmentss* for the first drop down. For the second drop down, we select *Yes* and decide to make compounds with only in silico generated parameters (hepatic clearance, fraction unbound in plasma) also available for this example. For the third item under the *Model* card, we have to upload a csv file with the nominal plasma in vitro bioactive concentration (uM) of each of the compounds we want to simulate. Therefore, we upload the following csv table. The csv file must have the exact format as the table below.

A csv file with bioactivity data for all ten chemicals. For the purpose of this example, the bioactivity data provided in this csv is fictional.

ChemicalName	CAS	BioactiveConcentration
Acetamiprid	135410-20-7	1.20
2,4-db	94-82-6	50.30
Acephate	30560-19-1	22.90
Abamectin	71751-41-2	82.60
Acetochlor	34256-82-1	0.95
Alachlor	15972-60-8	3.24
Aldicarb	116-06-3	5.90
Ametryn	834-12-8	13.60
Amitraz	33089-61-1	8.79
Atrazine	1912-24-9	17.65

Then, the fourth drop down menu asks the user whether they want to return a single OED per compound (a selected quantile) or all OED samples per compound. Since we want to output all generated OED samples, we select *Return all OED samples (will also return the 5th dose quantile)*. Thus, the completed *Model Specifications* tab should look like the two images below.

The screenshot shows the 'Model Specifications' tab of the Toxicokinetic Chemical Simulator (ToCS). The interface is divided into two main sections: 'DOSING' on the left and 'MODEL' on the right. The 'DOSING' section is currently empty, displaying the message 'No options for this category.' The 'MODEL' section contains several configuration options. At the top, a message states: 'species other than 'Human' is selected, '3compartments' must be chosen.' Below this, a dropdown menu is set to '3compartments'. Further down, a text block explains: 'Select whether to use in silico generated parameters for compounds with missing in vitro data. These parameters will not overwrite existing in vitro data, and it will expand the number of compounds available.' A dropdown menu below this text is set to 'Yes, load in silico parameters'. The next section instructs the user to 'Upload one CSV file with your species' in vitro bioactive concentrations (in uM units) for all compounds. It specifies that the CSV should have three column names in the following order: ChemicalName, CAS, BioactiveConcentration. Below this instruction, there are two buttons: 'Browse...' and 'SampleBioData_10Ch'. The 'SampleBioData_10Ch' button is highlighted in blue and has a status bar below it that says 'Upload complete'. The final section in the 'MODEL' tab asks the user to 'Select whether to return all oral equivalent dose (OED) samples for each compound or a selected quantile.' A dropdown menu below this text is set to 'Return all OED samples (will also return the 5th dose quantile)'.

The completed model specifications tab for example 2.

Compound Selection Tab

As with the first example, the first drop down menu under the *Preloaded Compounds* card has the user select a set of simulations assumptions to implement. See example 1 above for descriptions of the assumptions. Since we want to use the nominal concentration in vitro as bioactive and restrictive clearance (protein binding taking into account in liver clearance), we select NULL for the first drop down menu (note that we could have also selected Honda3 for the same result). This then results in a new box below containing a list of preloaded compounds that we can select from. We search for the same ten compounds that we uploaded bioactivity data for and see that they are all present in the preloaded list. Thus, we select those compounds and ignore the *Uploaded Compounds* card. The completed *Compound Selection* tab should look like the images below.

Toxicokinetic Chemical Simulator (ToCS)
General Parameters
Model Specifications
Compound Selection
Advanced (Optional) Parameters
Run Simulation

INSTRUCTIONS

Below is a downloadable folder containing information on upload file formatting if compounds to be analyzed are not included in the preloaded compounds list. The uploaded file must be formatted exactly how the 'SampleCSV' file is structured (keeping columns even if there is no data). The header descriptions of the CSV and the minimal data inputs required for the CSV are in the 'DataDescriptions' and 'RequiredData' PDFs, respectively.

[Uploaded Compound File Folder](#)

PRELOADED COMPOUNDS

Select an IVIVE assumption to implement. For any input nominal bioactive concentration in vitro, the Honda1 assumption is recommended. Leave on 'NULL' if no assumptions are to be applied. See the 'IVIVE Simulation Examples' vignette for the description of the below assumption categories.

NULL

Select any preloaded compounds. Search through the list by clicking on the box and scrolling or typing in a name. The list may not show all available compounds. Click on a compound to select it. You may select multiple.

94-82-6, 2,4-db
71751-41-2, Abamectin
30560-19-1, Acephate
135410-20-7, Acetamiprid
34256-82-1, Acetochlor
15972-60-8, Alachlor
116-06-3, Aldicarb
834-12-8, Ametryn
33089-61-1, Amitraz
1912-24-9, Atrazine

UPLOADED COMPOUNDS

Upload a CSV file of data for compounds not in the preloaded list (if desired). See the 'Instructions' panel to the left for directions on file formatting requirements.

Browse...
No file selected

The completed compound selection tab for example 2.

Advanced (Optional) Parameters Tab

To speed up the computation time of the program, let's change the number of Monte Carlo samples generated for each compound (under the *Model Conditions* card) from 1000 to 500. Then under the *Output Specification* card, we want to upload the following CSV file with chemical exposure data to the program:

A csv file with exposure estimate data (mg/kg BW/day) for all ten chemicals. These exposure estimates were gathered from the EPA CompTox Dashboard. NHANES estimates were used if available. Otherwise, the EPA's SEEM3 and SEEM2 model predictions were used.

ChemicalName	CAS	Upper	Median	Lower
Acetamiprid	135410-20-7	2.39e-04	5.99e-07	NA
2,4-db	94-82-6	1.29e-04	1.31e-06	NA
Acephate	30560-19-1	1.40e-06	3.37e-07	4.79e-08
Acetochlor	34256-82-1	2.00e-07	4.93e-08	4.74e-09
Alachlor	15972-60-8	1.40e-06	2.7e-07	NA
Aldicarb	116-06-3	2.39e-03	3.03e-06	NA
Ametryn	834-12-8	1.43e-03	2.23e-06	NA

ChemicalName	CAS	Upper	Median	Lower
Amitraz	33089-61-1	9.77e-04	1.24e-06	NA
Abamectin	71751-41-2	1.24e-05	4.17e-08	NA
Atrazine	1912-24-9	4.00e-07	5.76e-08	2.81e-09

For more details on the format of this file, please review the *Introduction to ToCS* vignette and/or the Exposure Data File Folder below the upload area in the interface. Also, since we want to output the plasma OED, we keep the second drop down menu as *plasma*. Thus, the completed *Advanced Parameters* tab should look like the image below.

Toxicokinetic Chemical Simulator (ToCS) General Parameters Model Specifications Compound Selection **Advanced (Optional) Parameters** Run Simulation

MODEL CONDITIONS

Enter the number of Monte Carlo samples generated for each compound.

500

Select which chemical concentration is treated as bioactive in vivo.

Total chemical concentration (default)

Enter the minimum acceptable chemical fraction unbound in presence of plasma proteins. All values below this will be set to this value.

0.0001

Select whether protein binding is taken into account in liver clearance.

Yes, include protein binding (default)

Select whether to adjust the chemical fraction unbound in presence of plasma proteins for lipid binding.

Yes, adjust the fraction of unbound plasma (default)

Select whether to use regressions when

MODEL SOLVER

No options for this category.

BIOAVAILABILITY

Enter a default value for the Caco-2 apical-to-basal membrane permeability (denoted Caco2.Pab, 10^{-6} cm/s).

1.6

Select whether to use the Caco2.Pab value set above to estimate F_{abs} (the in vivo measured fraction of an oral dose absorbed from the gut lumen into the gut) if bioavailability data is unavailable.

Use the Caco2.Pab value selected above (default)

Select whether to use the Caco2.Pab value set above to calculate F_{gut} (the in vivo measured fraction of an oral dose that passes gut metabolism and clearance) if bioavailability data is unavailable.

Use the Caco2.Pab value selected above (default)

Select whether to overwrite in vivo F_{abs} and F_{gut} data (if available).

Do not overwrite in vivo values

OUTPUT SPECIFICATION

Upload a CSV file of exposure data for the selected compounds. See the downloadable folder below for file upload formatting details.

Browse... SampleExpData_10CI

Upload complete

[Exposure Data File Folder](#)

Select the dose output units from either mg/kg BW/day (mg/kgpday) (default) or umol/kg BW/day (umol/kgpday).

mg/kgpday

Select the output concentration type. Selecting 'Tissue' for the 3compartmentss model will return the whole body plasma concentration.

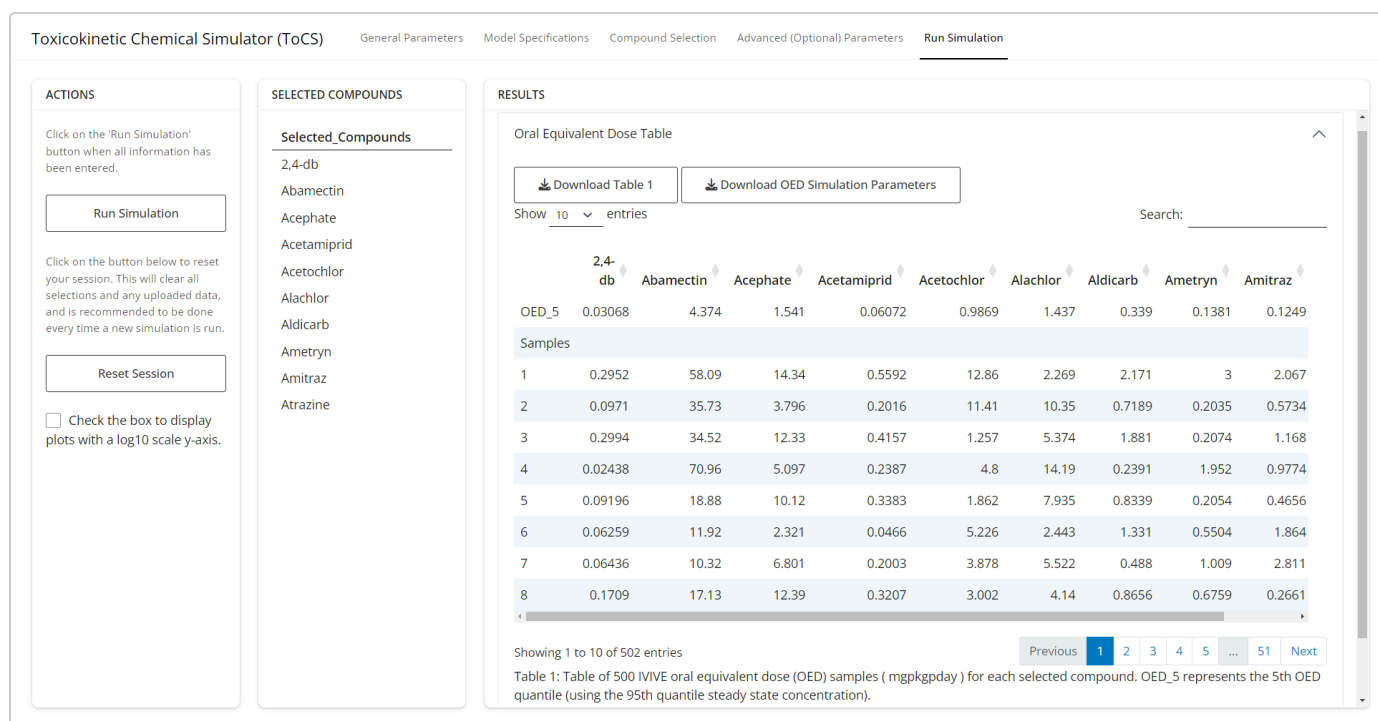
plasma

The completed advanced parameters tab for example 2.

Run Simulation Tab

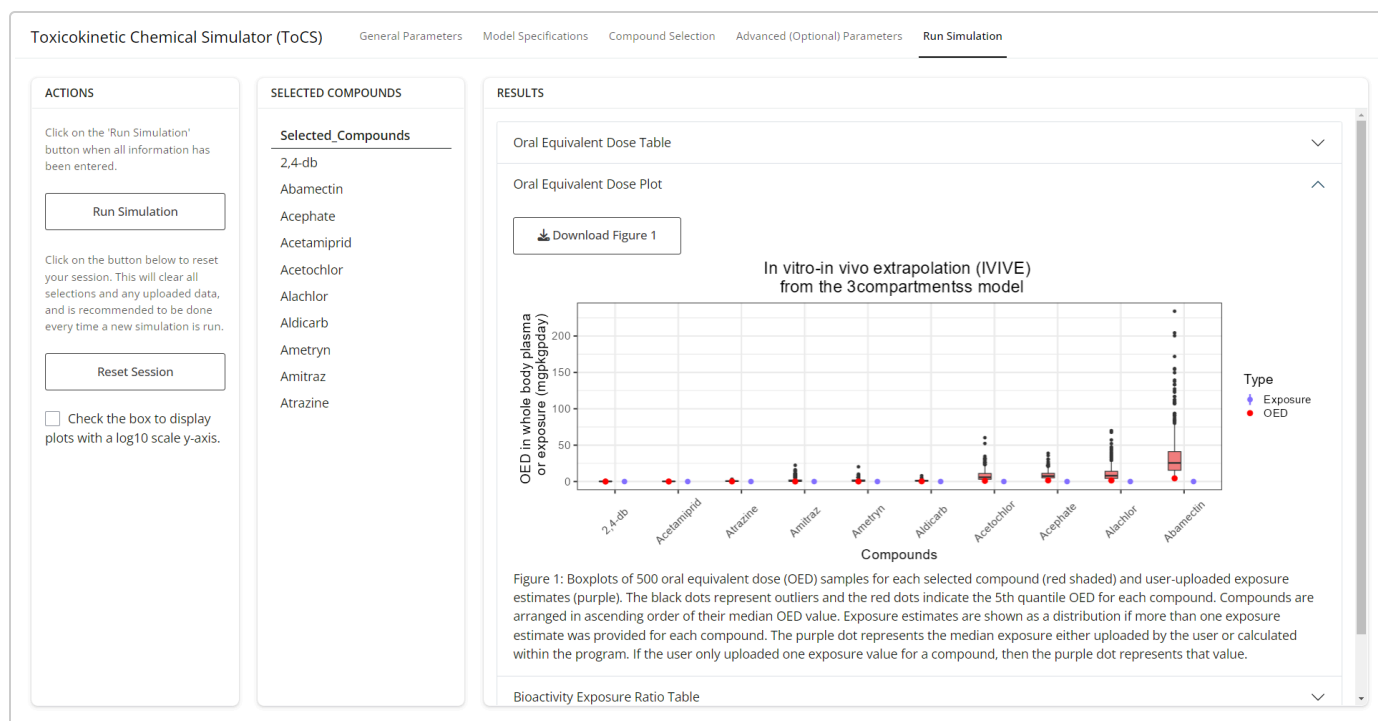
Now that all user parameter selections have been made and all compounds appear under the *Select Compounds* card, we click the *Run Simulation* button. Once the simulation is complete, the user's interface should look like the image below. The first tab shows a table of all generated OEDs for each compound (based on different steady state concentrations obtained from Monte Carlo simulations). The first row of the table contains the 5th quantile plasma OED, and then the rows below indicate the sample number and corresponding plasma OED from that sample. The user can view the various pages of samples by clicking the *Next* button at the bottom of the table. The table is available for download by the user if the user clicks the

Download Table 1 button. The user is also able to download all of the simulation parameters and chemical information used in the simulation by clicking the **Download OED Simulation Parameters** tab.



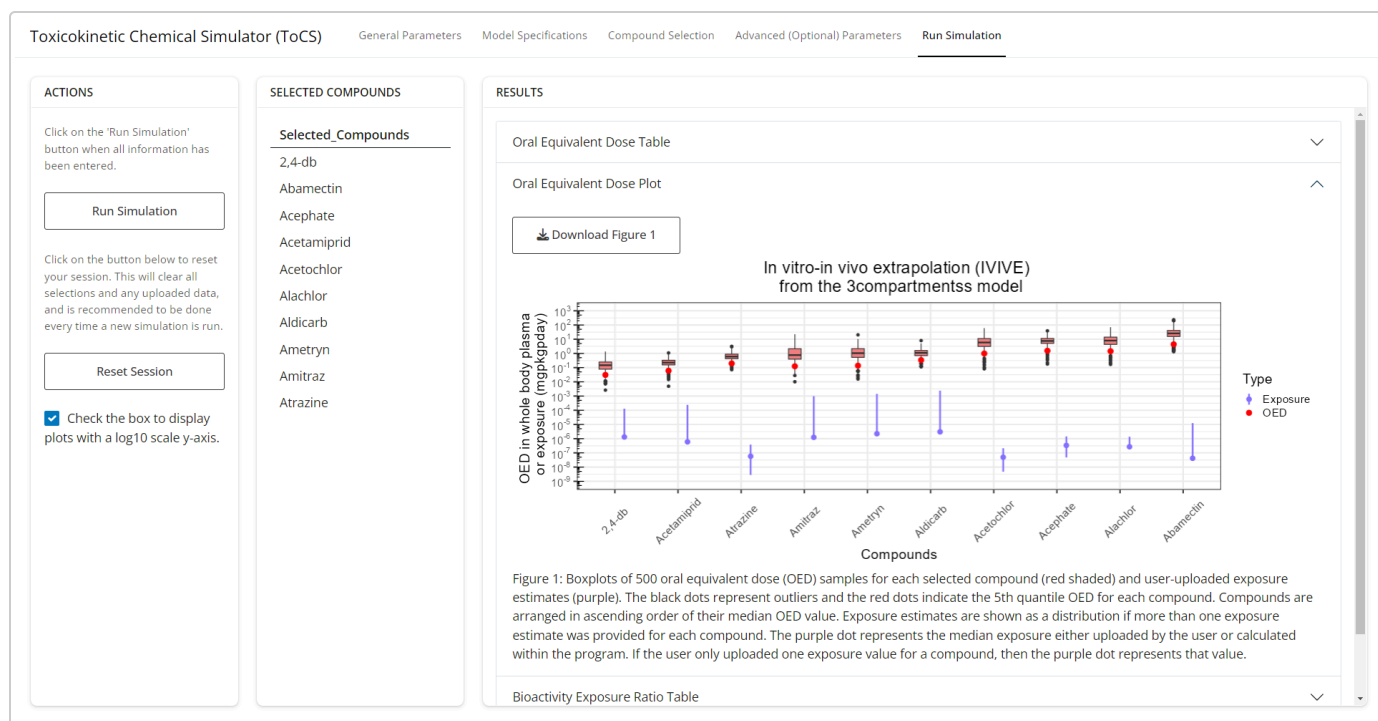
The completed run simulation tab for example 2 showing the expanded OED table tab.

The image below is the completed OED plot tab, which shows boxplots describing the distribution of all OED samples for each simulated compound with line ranges of exposure estimate distributions (in purple) next to each chemical (though they look like singular points due to the linear y-axis). The black dots are outlying samples, and the large red dots represent the 5th quantile OED from the OED table in the previous drop down tab. The plot shown below uses a linear y-axis, but as we can see, this makes it challenging to visually see the distribution of OED samples and all exposure estimates.



The completed run simulation tab for example 2 showing the expanded OED plot tab where the OED plot has a linear scale y-axis and showcases the OED distributions against the chemical exposure estimate distributions.

Therefore, we can click the box under the *Actions* card to change the scale of the y-axis to a log10 scale. That then results in the image shown below, which allows the user to clearly view and compare the OED sample distributions and exposure estimates of all compounds. The description of the exposure estimates is the same as in example 1. The user can download either the linear or log10 scale plot by clicking the *Download Figure 1* button.



The completed run simulation tab for example 2 showing the expanded OED plot tab where the plot has a log10 scale y-axis.

The table below shows the calculated bioactivity exposure ratios (BERs) for the chemicals included in the simulation with exposure data. The BER was calculated as the quotient of the 5th quantile OED (red dot from the OED plot) and the upper exposure estimate data point. Users can download the table by clicking the *Download Table 2* button above the table.

Toxicokinetic Chemical Simulator (ToCS) General Parameters Model Specifications Compound Selection Advanced (Optional) Parameters **Run Simulation**

ACTIONS

Click on the 'Run Simulation' button when all information has been entered.

Run Simulation

Click on the button below to reset your session. This will clear all selections and any uploaded data, and is recommended to be done every time a new simulation is run.

Reset Session

☒ Check the box to display plots with a log10 scale y-axis.

SELECTED COMPOUNDS

Selected_Compounds

2,4-db
Abamectin
Acephate
Acetamiprid
Acetochlor
Alachlor
Aldicarb
Ametryn
Amitraz
Atrazine

RESULTS

Oral Equivalent Dose Plot

Bioactivity Exposure Ratio Table

Download Table 2

Show **10** entries Search: _____

	CompoundName	BER
1	2,4-db	237.8
2	Abamectin	352700
3	Acephate	1070000
4	Acetamiprid	254.1
5	Acetochlor	4677000
6	Alachlor	1049000
7	Aldicarb	141.8
8	Ametryn	96.57
9	Amitraz	127.8
10	Atrazine	516500

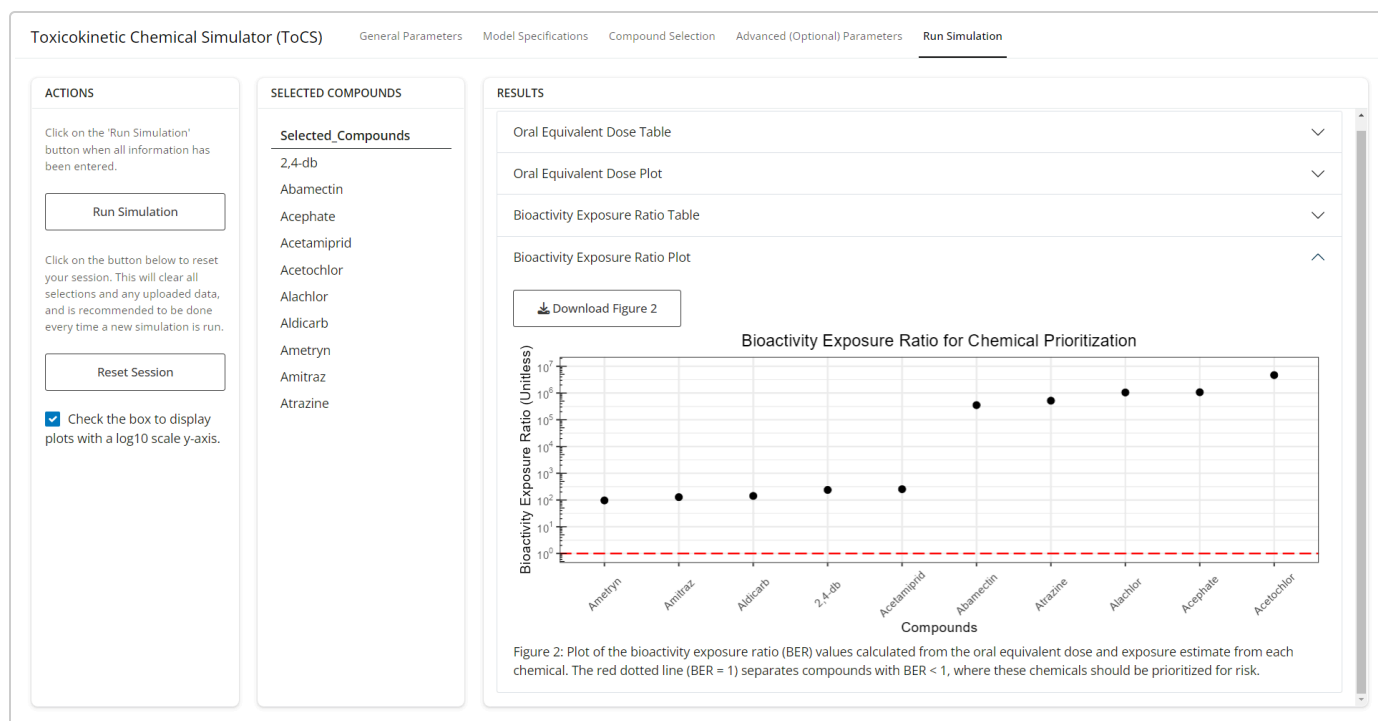
Showing 1 to 10 of 10 entries

Table 2: Table of the bioactivity exposure ratio (BER) for each selected compound.

Previous **1** Next

The completed run simulation tab for example 2 showing the expanded BER table tab.

The image below shows the final output of the IVIVE module which is a plot of the bioactivity exposure ratio (BER) for each chemical. As we can see from the plot, all BERs in this simulation are $\gg 1$ (visually seen by the dotted red line), so they are not considered a risk with the current data used in the simulation. Users can download this plot by clicking the *Download Figure 2* button above the plot.



The completed run simulation tab for example 2 showing the expanded BER plot tab where the plot has a log10 scale y-axis.

If we wanted to run another simulation, we would click the *Reset Session* button under the *Actions* button, which would clear all parameter inputs and simulations and return the interface to the *General Parameters* tab.

Example 3

Let's say that we want to perform human IVIVE to obtain 10th quantile liver OEDs using the 3compartment model. We will use the nominal bioactivity data for the eight compounds used in example 1, and want to include restrictive clearance in the model. We will not upload chemical exposure data for this example.

General Parameters Tab

Since we want to perform IVIVE, we select the *In vitro in vivo extrapolation (IVIVE)* option under the drop down menu from the *Output* card. Then under the *Species* card, we select *Human* for the first drop down and *Yes* for the second drop down as with other vignettes. Thus, the completed *General Parameters* tab should look like the image below.

Toxicokinetic Chemical Simulator (ToCS)
General Parameters
Model Specifications
Compound Selection
Advanced (Optional) Parameters
Run Simulation

INSTRUCTIONS

Fill out the prompts on each of the above tabs moving left to right. Then, click the 'Run Simulation' tab to run the simulation or reset all selections.

ToCS provides four toxicokinetic (TK) outputs: 1) Concentration-time profiles, which returns chemical concentrations in bodily compartments over time, 2) Steady state (SS) concentrations, which returns SS concentrations in bodily compartments from an oral infusion, 3) In vitro in vivo extrapolation (IVIVE), which returns oral equivalent doses to in vitro bioactive concentrations, 4) Parameter calculations, which returns elimination rates, volumes of distribution, tissue to unbound plasma partition coefficients, half-lives, and total plasma clearances.

This application uses the U.S. EPA's R package 'httk'. For more information on 'httk', refer to <https://doi.org/10.18637/jss.v079.i04> and/or <https://cran.r-project.org/web/packages/httk>

For additional guidance on ToCS, please refer to the vignettes (<https://github.com/KristenWindoloski/ToCS/tree/main/vignettes>). To report issues or suggestions for improvement, visit <https://github.com/KristenWindoloski/ToCS/issues>.

OUTPUT

Select the desired output.

In vitro in vivo extrapolation (IVIVE) ▾

SPECIES

Select the species to analyze.

Human ▾

Do you want to use human in vitro data if in vitro data for the selected species is missing?

Yes ▾

The completed General Parameters tab for example 3.

Model Specifications Tab

Under the *Dosing* card, there are no options to select from. Under the *Model* card, we select *3compartment* for the first drop down. For the second drop down, we select *No* and decide to make compounds with only in vitro data available for this example. For the third item under the *Model* card, we have to upload a csv file with the nominal plasma in vitro bioactive concentrations of the eight compounds we want to simulate. Therefore, we upload the following csv table. Note that the table must have the following format.

A csv file with bioactivity data for all eight chemicals. For the purpose of this example, the bioactivity data provided in this csv is fictional.

ChemicalName	CAS	BioactiveConcentration
Acetamiprid	135410-20-7	1.20
2,4-db	94-82-6	50.30
Acephate	30560-19-1	22.90
Acetochlor	34256-82-1	0.95
Alachlor	15972-60-8	3.24
Aldicarb	116-06-3	5.90

ChemicalName	CAS	BioactiveConcentration
Ametryn	834-12-8	13.60
Amitraz	33089-61-1	8.79

Then, the fourth drop down menu asks the user whether they want to return a single OED per compound (a selected quantile) or all OED samples per compound. Since we want to output the 10th quantile OED, we select *Only return a specified dose quantile (default)*. This results in the appearance of another input box. In this final box under the *Model* card, we enter the steady state concentration quantile that we desire to use in our OED calculation (0.90). This will return the 10th quantile OED in the simulation results. Thus, the completed *Model Specifications* tab should look like the two images below.

Toxicokinetic Chemical Simulator (ToCS)
General Parameters
Model Specifications
Compound Selection
Advanced (Optional) Parameters
Run Simulation

DOSING
No options for this category.

MODEL
Select the model to simulate. If a species other than 'Human' is selected, '3compartments' must be chosen.
3compartment
Select whether to use in silico generated parameters for compounds with missing in vitro data. These parameters will not overwrite existing in vitro data, and it will expand the number of compounds available.
No, do not load in silico parameters
Upload one CSV file with your species' in vitro bioactive concentrations (in uM units) for all compounds. The CSV should have three column names in the following order with one row per chemical: ChemicalName, CAS, BioactiveConcentration.
Browse...
SampleBioData_8Che
Upload complete
Select whether to return all oral equivalent dose (OED) samples for each compound or a selected quantile.
Only return a specified dose quantile

The completed upper portion of the model specifications tab for example 3.

Toxicokinetic Chemical Simulator (ToCS) General Parameters **Model Specifications** Compound Selection Advanced (Optional) Parameters Run Simulation

DOSING

No options for this category.

MODEL

parameters will not overwrite existing in vitro data, and it will expand the number of compounds available.

No, do not load in silico parameters ▾

Upload one CSV file with your species' in vitro bioactive concentrations (in uM units) for all compounds. The CSV should have three column names in the following order with one row per chemical: ChemicalName, CAS, BioactiveConcentration.

Browse...
SampleBioData_8Che

Upload complete

Select whether to return all oral equivalent dose (OED) samples for each compound or a selected quantile.

Only return a specified dose quantile (default) ▾

Enter the steady state concentration quantile (as a decimal) to be used in the OED calculation. Selecting the 95th concentration quantile will output the 5th OED quantile.

0.9

The completed lower portion of the model specifications tab for example 3.

Compound Selection Tab

As with the first example, the first drop down menu under the *Preloaded Compounds* card has the user select a set of simulations assumptions to implement. See example 1 for descriptions of the assumptions. As with example 2, we will select NULL for the first drop down menu since we wanted to use 1) the nominal in vitro concentration as bioactive, 2) restrictive clearance, and 3) liver tissue as bioactive in vivo. This then results in a new box below containing a list of preloaded compounds that we can select from. We search for the same eight compounds that we uploaded bioactivity data for and see that they are all present in the preloaded list. Thus, we select those compounds and ignore the *Uploaded Compounds* card. The completed *Compound Selection* tab should look like the images below.

Toxicokinetic Chemical Simulator (ToCS)
General Parameters
Model Specifications
Compound Selection
Advanced (Optional) Parameters
Run Simulation

INSTRUCTIONS

Below is a downloadable folder containing information on upload file formatting if compounds to be analyzed are not included in the preloaded compounds list. The uploaded file must be formatted exactly how the 'SampleCSV' file is structured (keeping columns even if there is no data). The header descriptions of the CSV and the minimal data inputs required for the CSV are in the 'DataDescriptions' and 'RequiredData' PDFs, respectively.

[Uploaded Compound File Folder](#)

PRELOADED COMPOUNDS

Select an IVIVE assumption to implement. For any input nominal bioactive concentration in vitro, the Honda1 assumption is recommended. Leave on 'NULL' if no assumptions are to be applied. See the 'IVIVE Simulation Examples' vignette for the description of the below assumption categories.

NULL

Select any preloaded compounds. Search through the list by clicking on the box and scrolling or typing in a name. The list may not show all available compounds. Click on a compound to select it. You may select multiple.

94-82-6, 2,4-db

30560-19-1, Acephate

135410-20-7, Acetamiprid

34256-82-1, Acetochlor

15972-60-8, Alachlor

116-06-3, Aldicarb

834-12-8, Ametryn

33089-61-1, Amitraz

UPLOADED COMPOUNDS

Upload a CSV file of data for compounds not in the preloaded list (if desired). See the 'Instructions' panel to the left for directions on file formatting requirements.

Browse...
No file selected

The completed compound selection tab for example 3.

Advanced (Optional) Parameters Tab

Since we want to output the OED in the liver, we select *tissue* under the second drop down menu in the *Output Specification* card specifying the output concentration type. This prompts the appearance of a third drop down menu. We select *liver* from this menu since we want the liver OED. No changes to other parameters on this page should be made for this example. Thus, the completed *Advanced Parameters* tab should look like the image below.

Toxicokinetic Chemical Simulator (ToCS)
General Parameters
Model Specifications
Compound Selection
Advanced (Optional) Parameters
Run Simulation

MODEL CONDITIONS

Enter the number of Monte Carlo samples generated for each compound.

Enter the minimum acceptable chemical fraction unbound in presence of plasma proteins. All values below this will be set to this value.

Select whether protein binding is taken into account in liver clearance.

Select whether to adjust the chemical fraction unbound in presence of plasma proteins for lipid binding.

Select whether to use regressions when calculating partition coefficients.

MODEL SOLVER

No options for this category.

BIOAVAILABILITY

Enter a default value for the Caco-2 apical-to-basal membrane permeability (denoted Caco2.Pab, 10^{-6} cm/s).

Select whether to use the Caco2.Pab value set above to estimate F_{abs} (the in vivo measured fraction of an oral dose absorbed from the gut lumen into the gut) if bioavailability data is unavailable.

Select whether to use the Caco2.Pab value set above to calculate F_{gut} (the in vivo measured fraction of an oral dose that passes gut metabolism and clearance) if bioavailability data is unavailable.

Select whether to overwrite in vivo F_{abs} and F_{gut} data (if available).

OUTPUT SPECIFICATION

Upload a CSV file of exposure data for the selected compounds. See the downloadable folder below for file upload formatting details.

[Exposure Data File Folder](#)

Select the dose output units from either mg/kg BW/day (mgpkpday) (default) or umol/kg BW/day (umolpkpday).

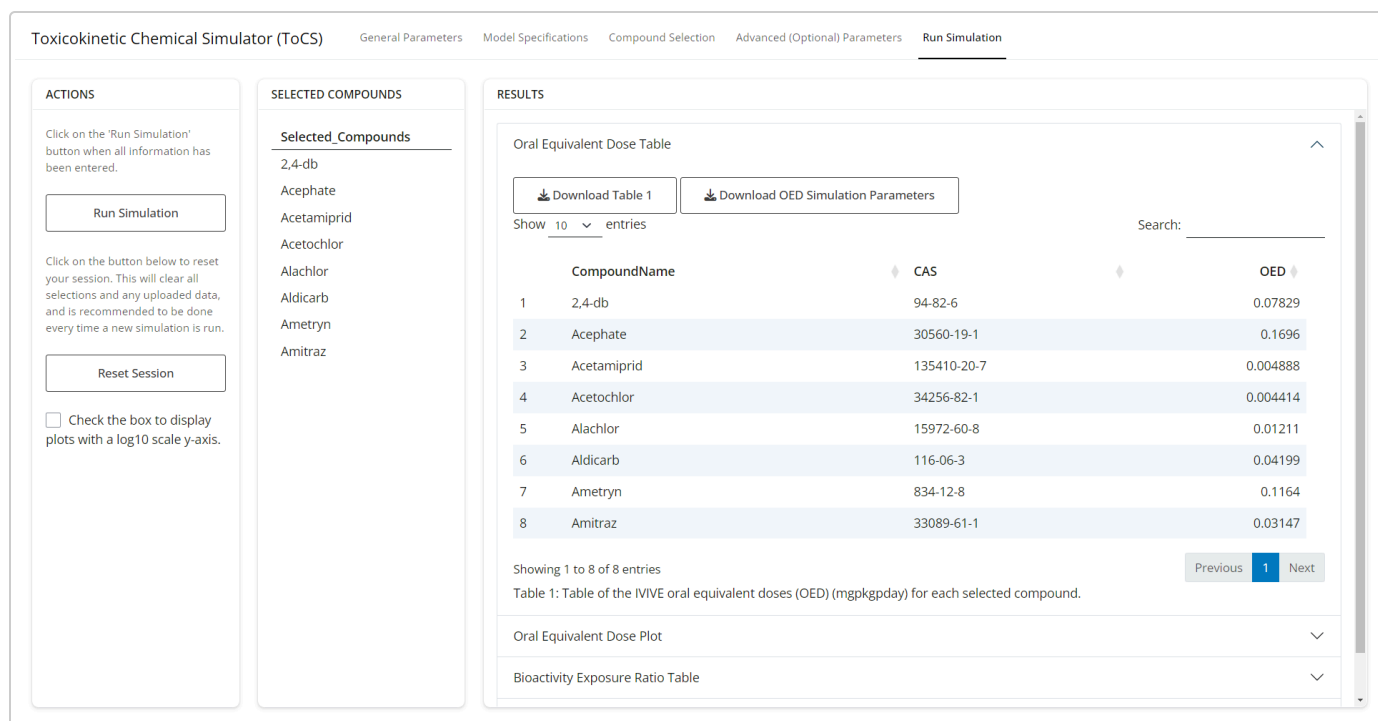
Select the output concentration type. Selecting 'Tissue' for the 3compartmentss model will return the whole body plasma concentration.

Select a tissue you want the output concentration in. Leave on 'NULL' if the whole body concentration is desired.

The completed advanced parameters tab for example 3.

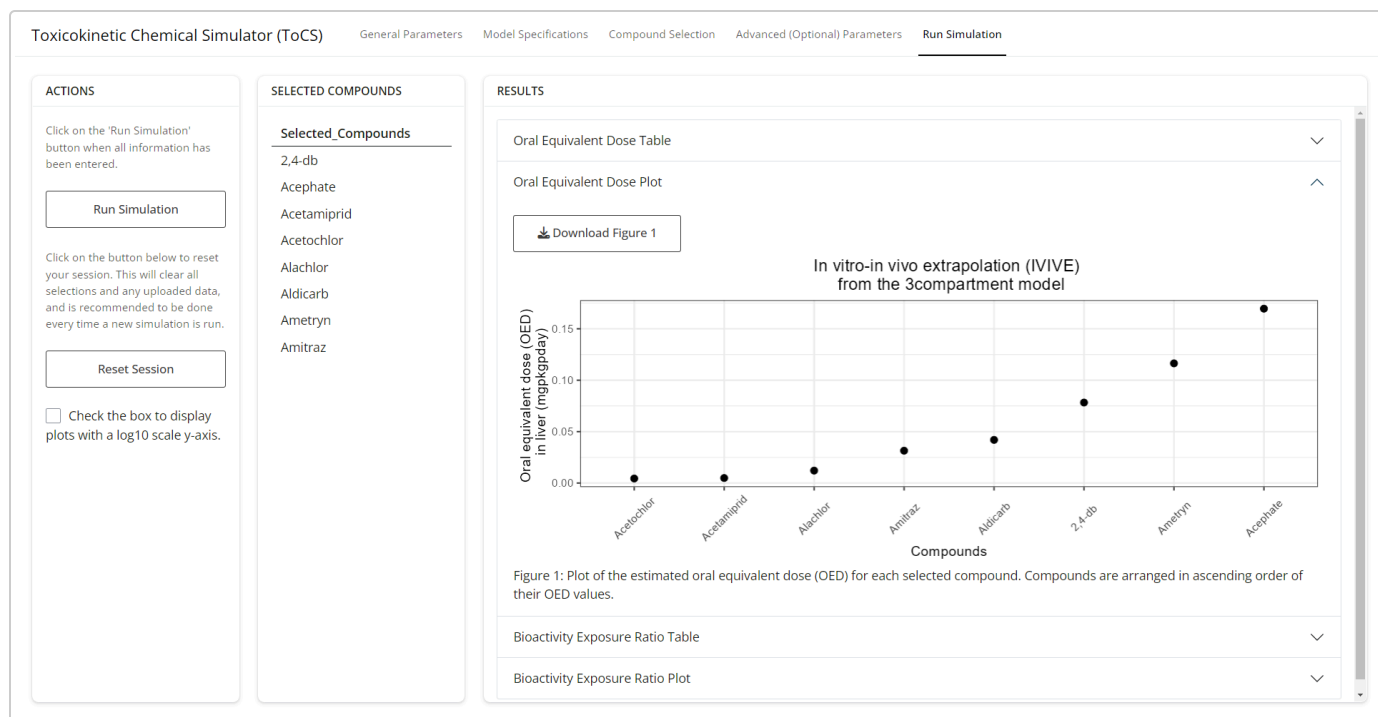
Run Simulation Tab

Now that all user parameter selections have been made and all compounds appear under the *Select Compounds* card, we click the *Run Simulation* button. Once the simulation is complete, the user's interface should look like the image below. The first tab shows a table of the OED for each compound. The table is available for download by the user if the user clicks the *Download Table 1* button. The user is also able to download all of the simulation parameters and chemical information used in the simulation by clicking the *Download OED Simulation Parameters* tab.



The completed run simulation tab for example 3 showing the expanded OED table tab.

The image below is the completed OED plot tab, which shows a plot of the 10th quantile OEDs using the liver steady state concentration for OED calculation. The plot shown below uses a linear y-axis, but as we can see, this makes it challenging to visually notice the magnitude of smaller OEDs.



The completed run simulation tab for example 3 showing the expanded OED plot tab where the plot has a linear scale y-axis.

Therefore, we can click the box under the *Actions* card to change the scale of the y-axis to a log10 scale. That then results in the image shown below, which allows the user to view the differences in magnitudes of all OEDs. The user can download either the linear or log10 scale plot by clicking the *Download Figure 1* button.



The completed run simulation tab for example 3 showing the expanded OED plot tab where the plot has a log10 scale y-axis.

The final two tabs on the IVIVE module exhibit the bioactivity exposure ratio (BER), if applicable for the simulation. Since no chemical exposure data was uploaded under the *Advanced Parameters* tab, no BERs were calculated for this simulation. If the user wanted to calculate BERs for this simulation, they would need to upload chemical exposure under the previous tab.

