

IVIVE Simulation Examples

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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. 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 shows the opening interface of the Toxicokinetic Chemical Simulator (ToCS) app. The interface has 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. Below the navigation bar, there are three main panels:

- INSTRUCTIONS:** This panel contains text explaining how to use the app. 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 provided by ToCS: 1) Concentration-time profiles, 2) Steady state (SS) concentrations, 3) In vitro in vivo extrapolation (IVIVE), and 4) Parameter calculations. It mentions that the app uses the U.S. EPA's R package 'httk' and provides links for more information. Finally, it refers to vignettes for additional guidance and a GitHub page for reporting issues or suggestions.
- OUTPUT:** This panel has a label "Select the desired output." and a dropdown menu with the text "Select".
- SPECIES:** This panel has a label "Select the species to analyze." and a dropdown menu with the text "Select". 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?" with another dropdown menu with the text "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.

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 has a top navigation bar with tabs: 'Toxicokinetic Chemical Simulator (ToCS)', 'General Parameters' (active), 'Model Specifications', 'Compound Selection', 'Advanced (Optional) Parameters', and 'Run Simulation'. The main content area is divided into three panels:

- INSTRUCTIONS:** Contains text about filling out prompts and moving tabs, a list of 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 also mentions the application uses the U.S. EPA's R package 'httk' and provides links for more information and reporting issues.
- OUTPUT:** Has a heading 'Select the desired output.' and a dropdown menu currently set to 'In vitro in vivo extrapolation (IVIVE)'.
- SPECIES:** Has a heading 'Select the species to analyze.' and a dropdown menu set to 'Human'. Below this is a question 'Do you want to use human in vitro data if in vitro data for the selected species is missing?' with a dropdown menu 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	Bioactive.Concentration
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.

The screenshot shows the 'Model Specifications' tab in 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 contains a message: 'No options for this category.' The 'MODEL' section contains several configuration options. At the top, it states: 'parameters will not overwrite existing in vitro data, and it will expand the number of compounds available.' Below this is a dropdown menu set to 'No, do not load in silico parameters'. Next is a text area for uploading a CSV file with species' in vitro bioactive concentrations, followed by a 'Browse...' button and a 'SampleBioData_8Che' button. Below these is an 'Upload complete' button. Then, there is a section for selecting the return of oral equivalent dose (OED) samples, with a dropdown menu set to 'Only return a specified dose quantile (default)'. Finally, there is a section for entering the steady state concentration quantile, with a text input field containing '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, ignore the *Uploaded Compounds* card, and click the *Load Compounds* button under the *Instructions* 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

Once you have chosen all compounds to analyze, click 'Load Compounds'.

Load Compounds

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

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

We omit changing any advanced parameter options for this example and leave all parameter values as the default (image shown below). The user can proceed to the next 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 (default)

OUTPUT SPECIFICATION

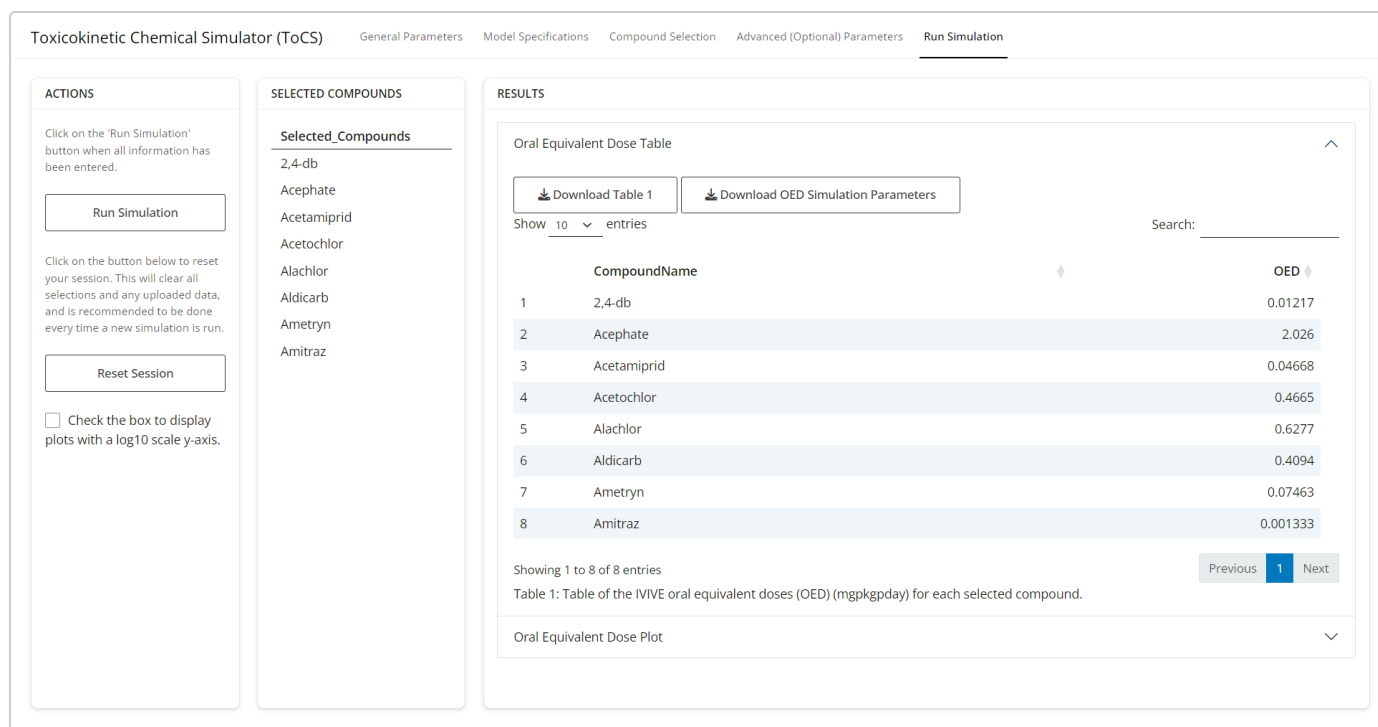
Select the dose output units from either mg/kg BW/day (mgpkgpday) (default) or umol/kg BW/day (umolpkgpday).

mgpkgpday

The completed advanced parameters tab for example 1.

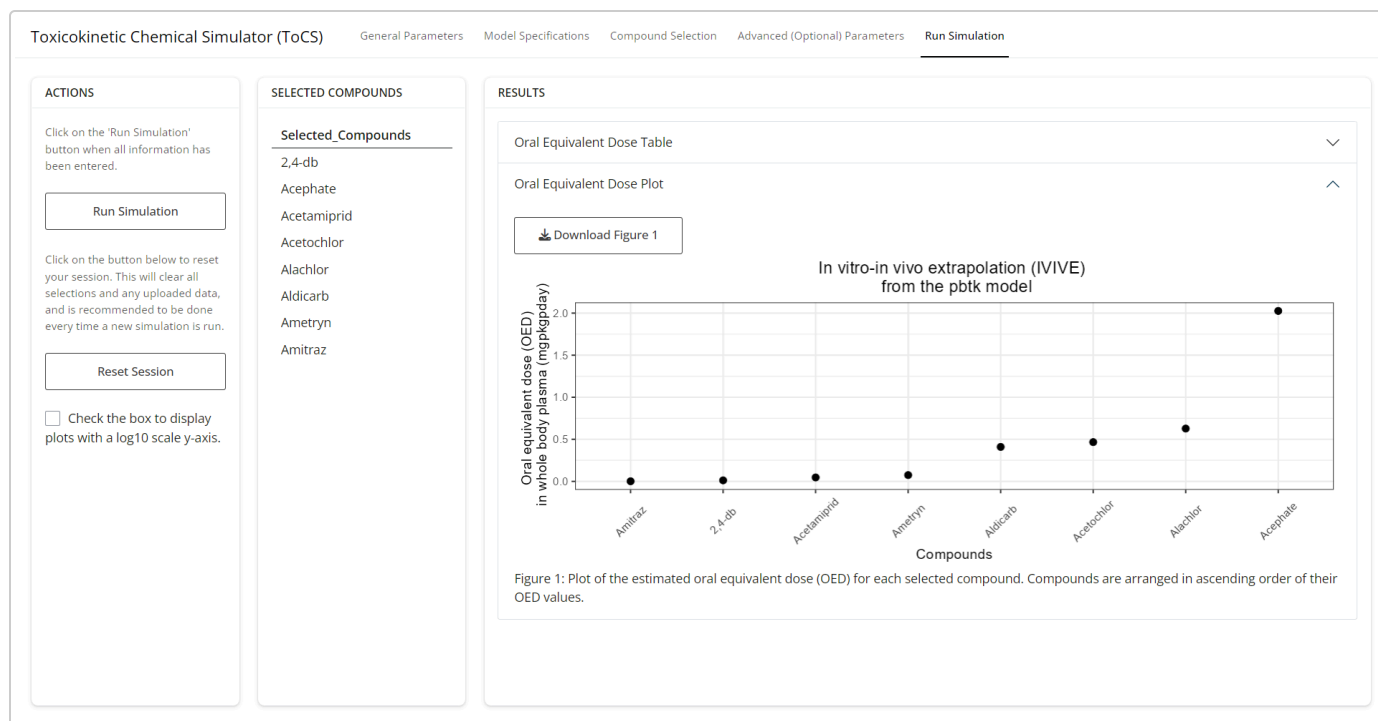
Run Simulation Tab

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 for each compound plotted in ascending order. 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.



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. 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.

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.

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 ParametersModel SpecificationsCompound SelectionAdvanced (Optional) ParametersRun 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 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	Bioactive.Concentration
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

ChemicalName	CAS	Bioactive.Concentration
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.

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

DOSING

No options for this category.

MODEL

species other than 'Human' is selected, '3compartmentss' must be chosen.

3compartmentss

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.

Yes, 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_10Ch

Upload complete

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

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, ignore the *Uploaded Compounds* card, and click the *Load Compounds* button under the *Instructions* card. The completed *Compound Selection* tab should look like the images below.

The screenshot shows the 'Compound Selection' tab of the Toxicokinetic Chemical Simulator (ToCS). The interface is divided into three main panels:

- INSTRUCTIONS:** Contains a 'Load Compounds' button and a link to the 'Uploaded Compound File Folder'.
- PRELOADED COMPOUNDS:** Features a dropdown menu set to 'NULL' and a list of ten compounds: 135410-20-7, Acetamiprid; 94-82-6, 2,4-db; 30560-19-1, Acephate; 71751-41-2, Abamectin; 34256-82-1, Acetochlor; 15972-60-8, Alachlor; 116-06-3, Aldicarb; 834-12-8, Ametryn; 33089-61-1, Amitraz; and 1912-24-9, Atrazine.
- UPLOADED COMPOUNDS:** Includes a 'Browse...' button and a 'No file selected' status.

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, 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 calculating partition coefficients.

MODEL SOLVER

No options for this category.

BIOAVAILABILITY

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 (default)

Select whether to keep F_{abs} and F_{gut} at 100% availability (which overwrites all other bioavailability parameter settings above).

Do not keep Fabs and Fgut at 100% availability (default)

OUTPUT SPECIFICATION

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

mgpkgsday

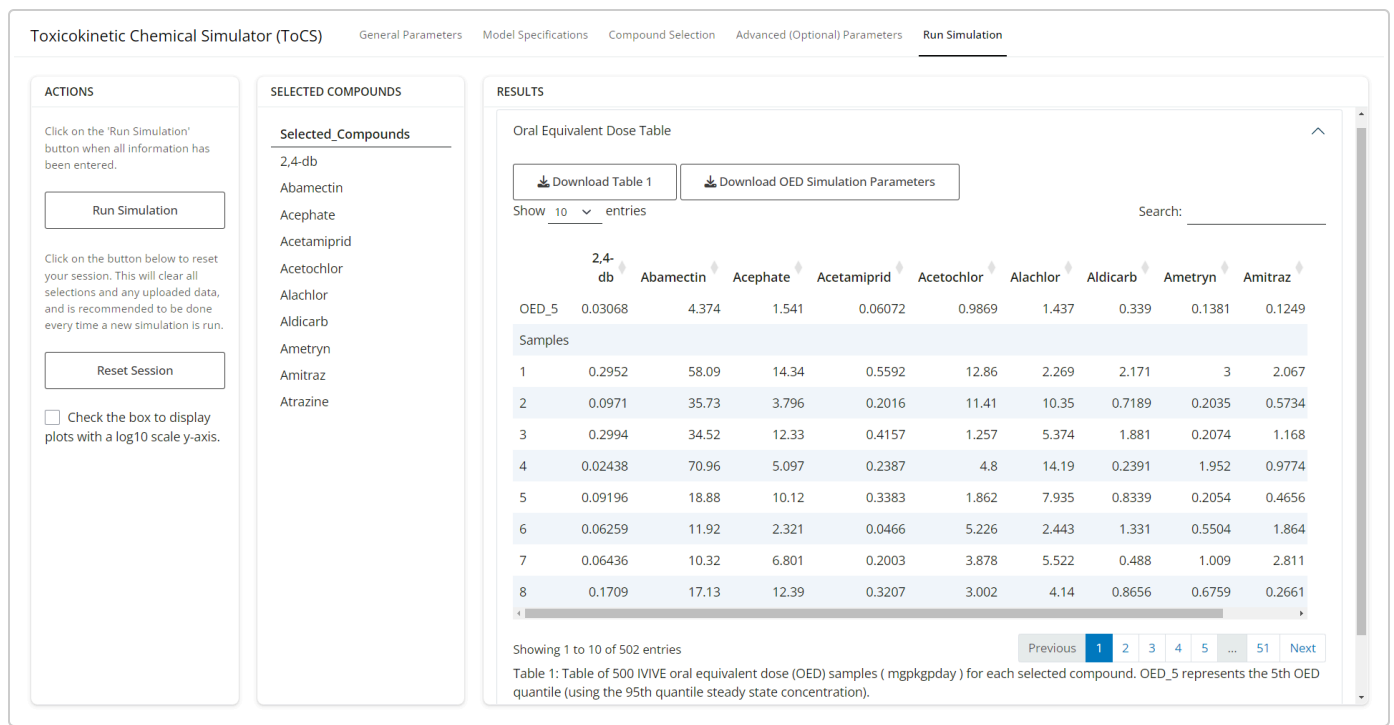
Select the output concentration type. Selecting 'Tissue' for the 3compartmentss model will return the whole body plasma concentration.

plasma

The completed advanced parameters tab for example 2.

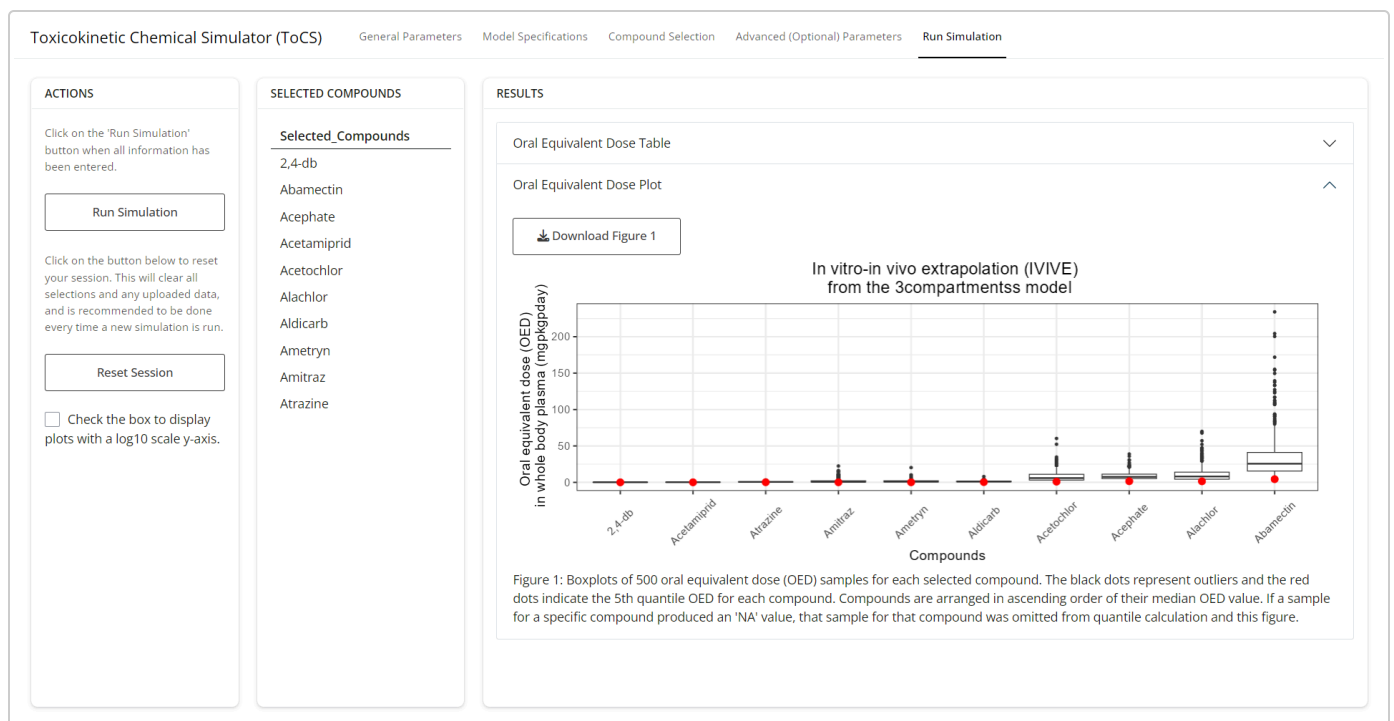
Run Simulation Tab

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.



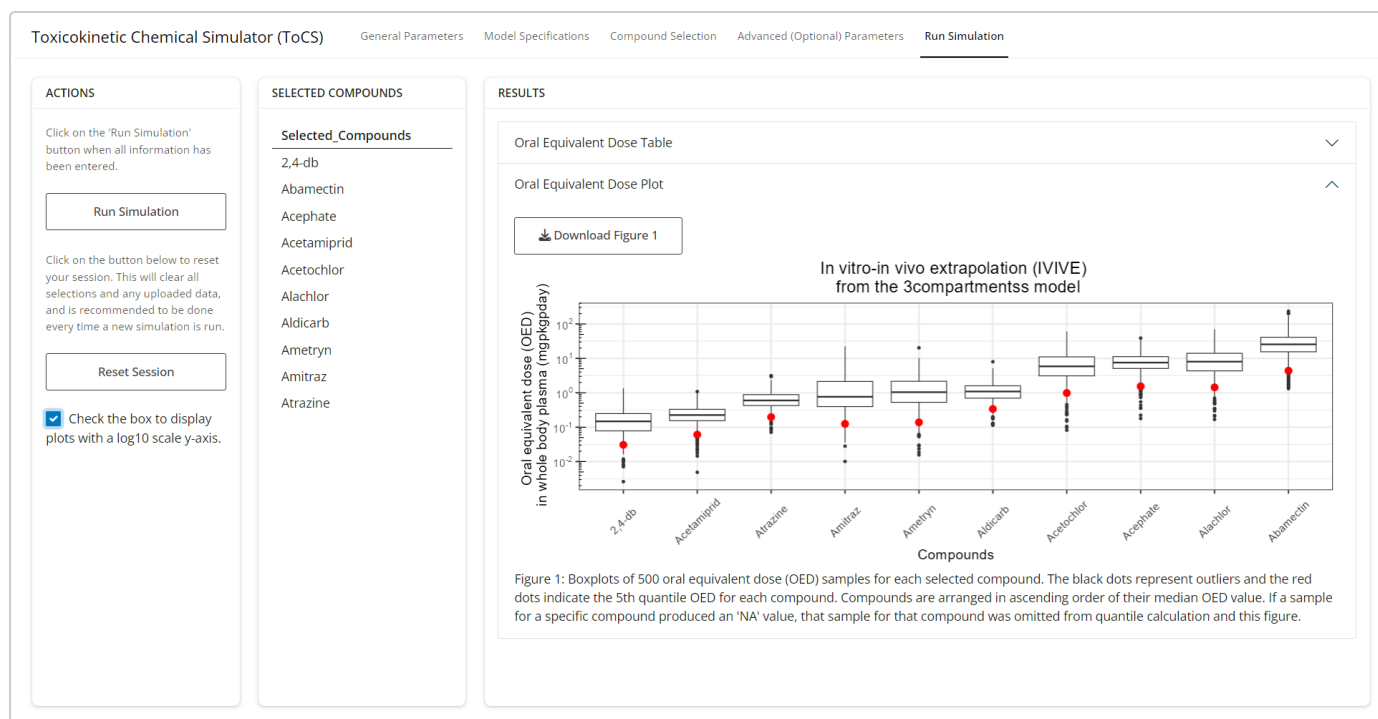
The completed run simulation tab for example 2 showing the expanded OED table tab.

The image below is the completed OED plot tab, which shows boxplots describing the distribution of all OED samples for each simulated compound. 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 samples for smaller OEDs.



The completed run simulation tab for example 2 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 OED sample distributions of all compounds. 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.

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.

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 select *No* for the second drop down menu under the *Species* card. 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 *3compartments* 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	Bioactive.Concentration
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	Bioactive.Concentration
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, ignore the *Uploaded Compounds* card, and click the *Load Compounds* button under the *Instructions* 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

Once you have chosen all compounds to analyze, click 'Load Compounds'.

Load Compounds

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

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

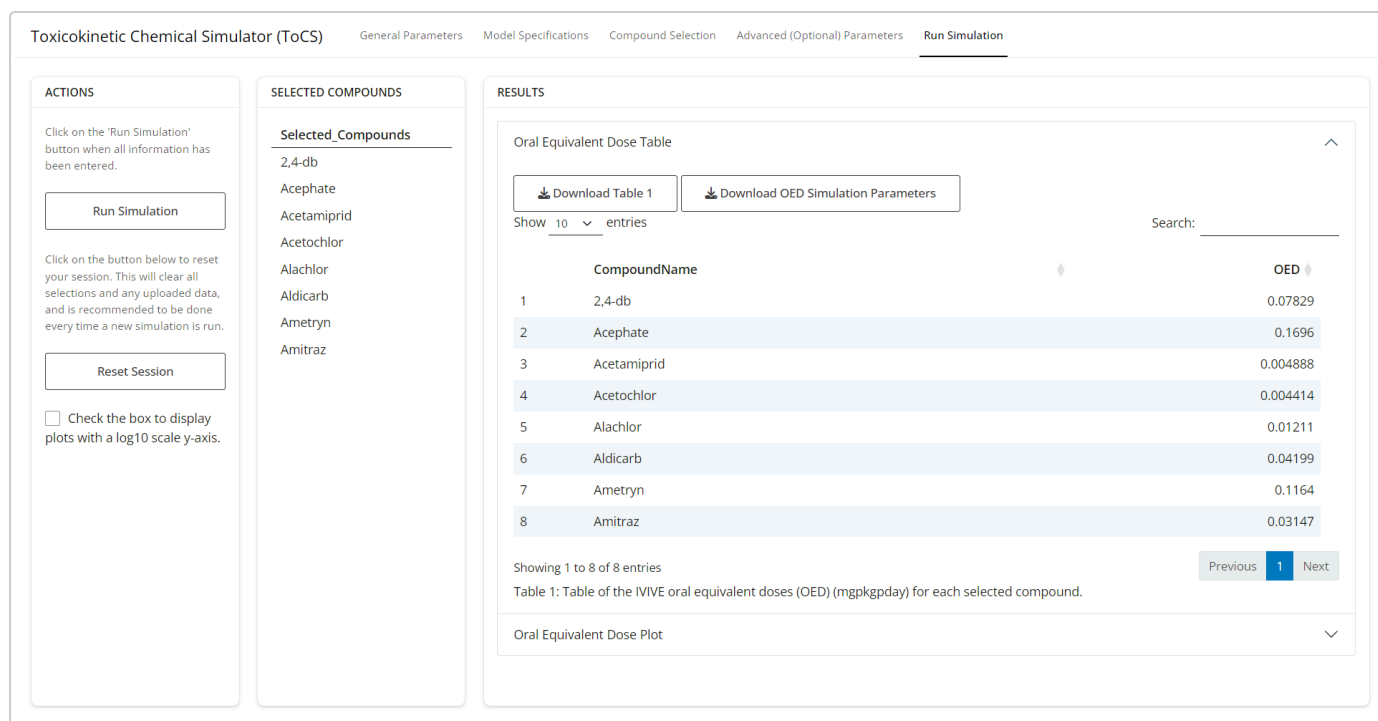
Select the output concentration type. Selecting 'Tissue' for the 3compartmentss model will return the whole body plasma concentration.

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.

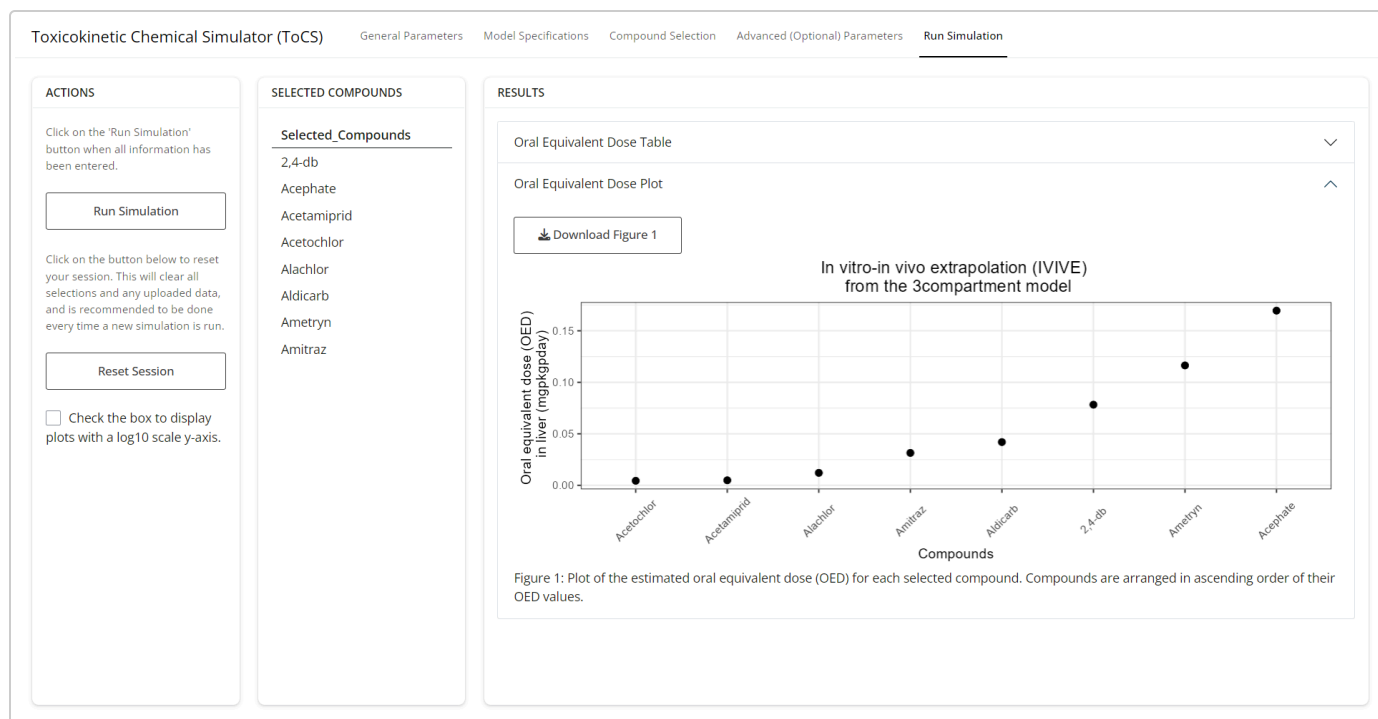
Run Simulation Tab

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.



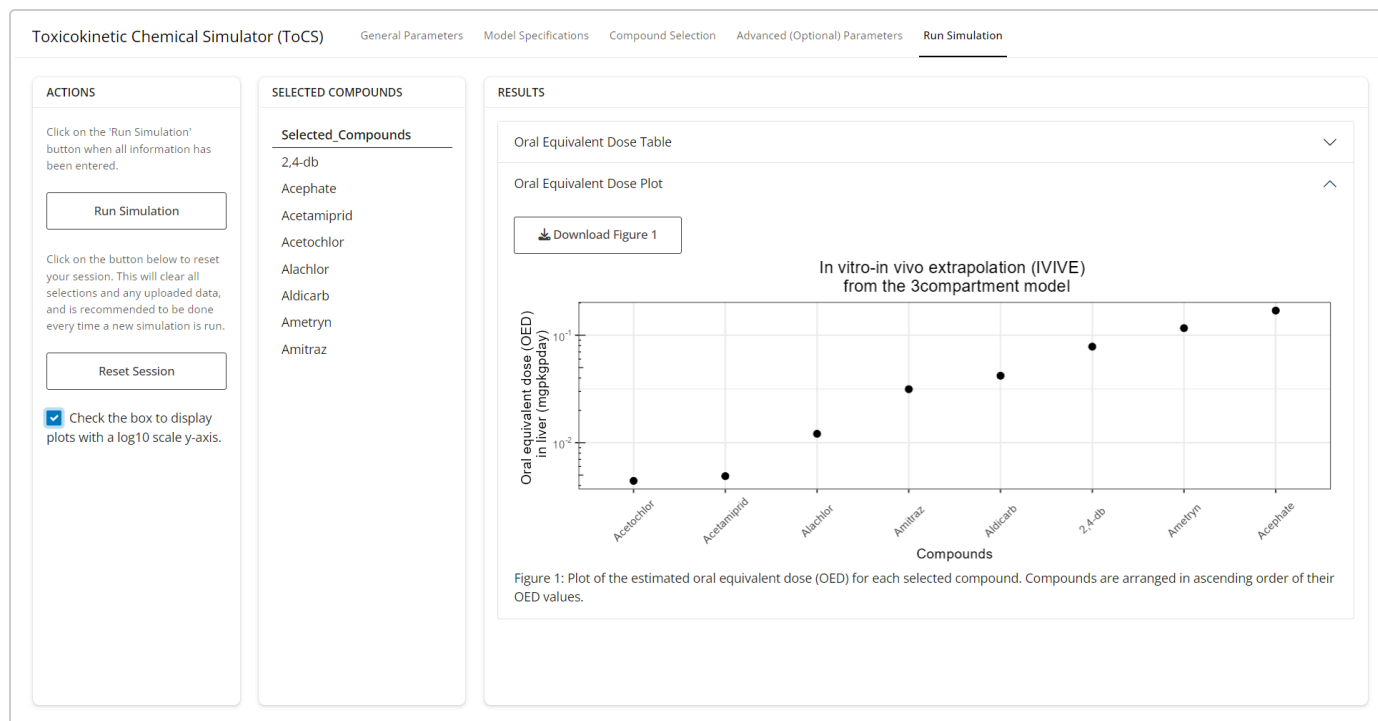
The completed run simulation tab for example 3 showing the expanded OED table tab.

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.

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.