IVIVE Simulation Examples

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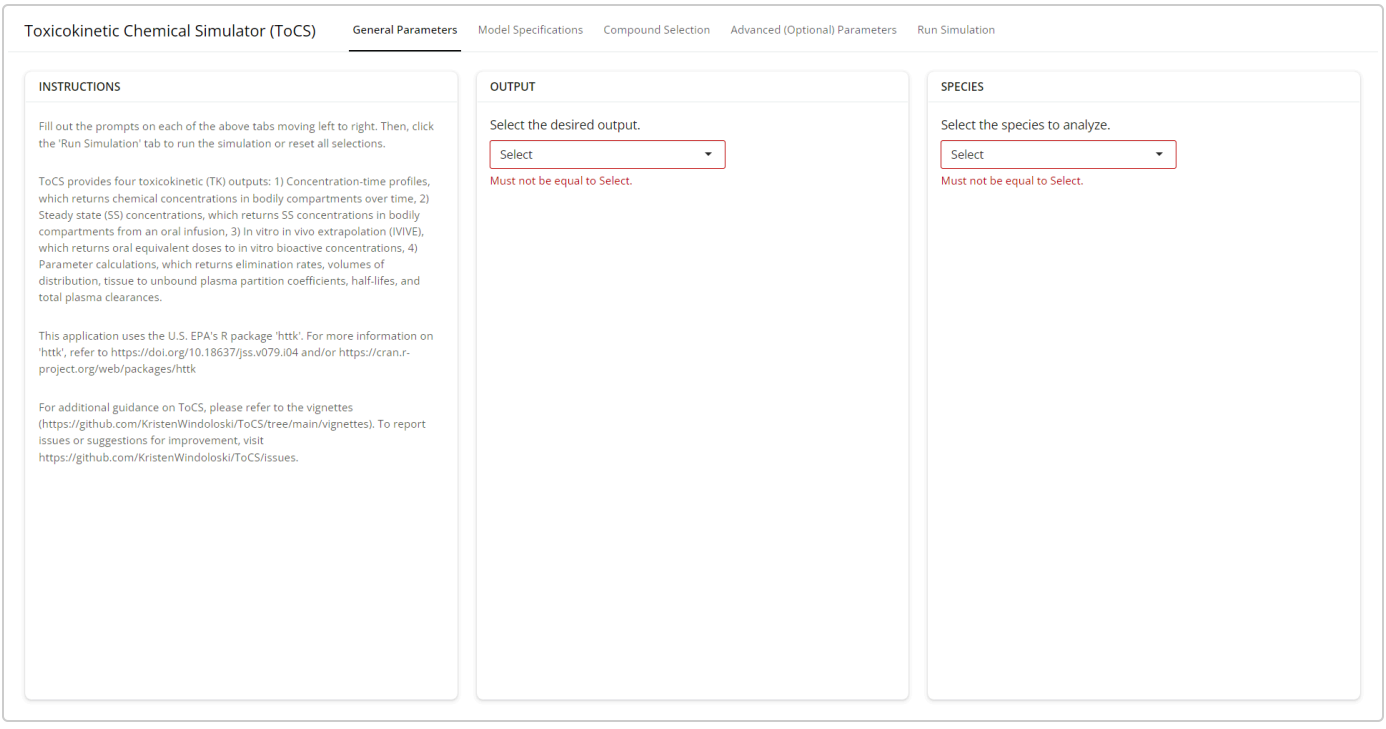
If you have not already read the *Introduction to the ToCS App* vignette, it is highly recommended to do so to get a general idea of the app's layout and obtain a detailed description of common user inputs across all output modules. Users should also review the README file on the ToCS GitHub page (github.com/KristenWindoloski/ToCS) 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. Two of the three examples also incorporate chemical exposure data 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.



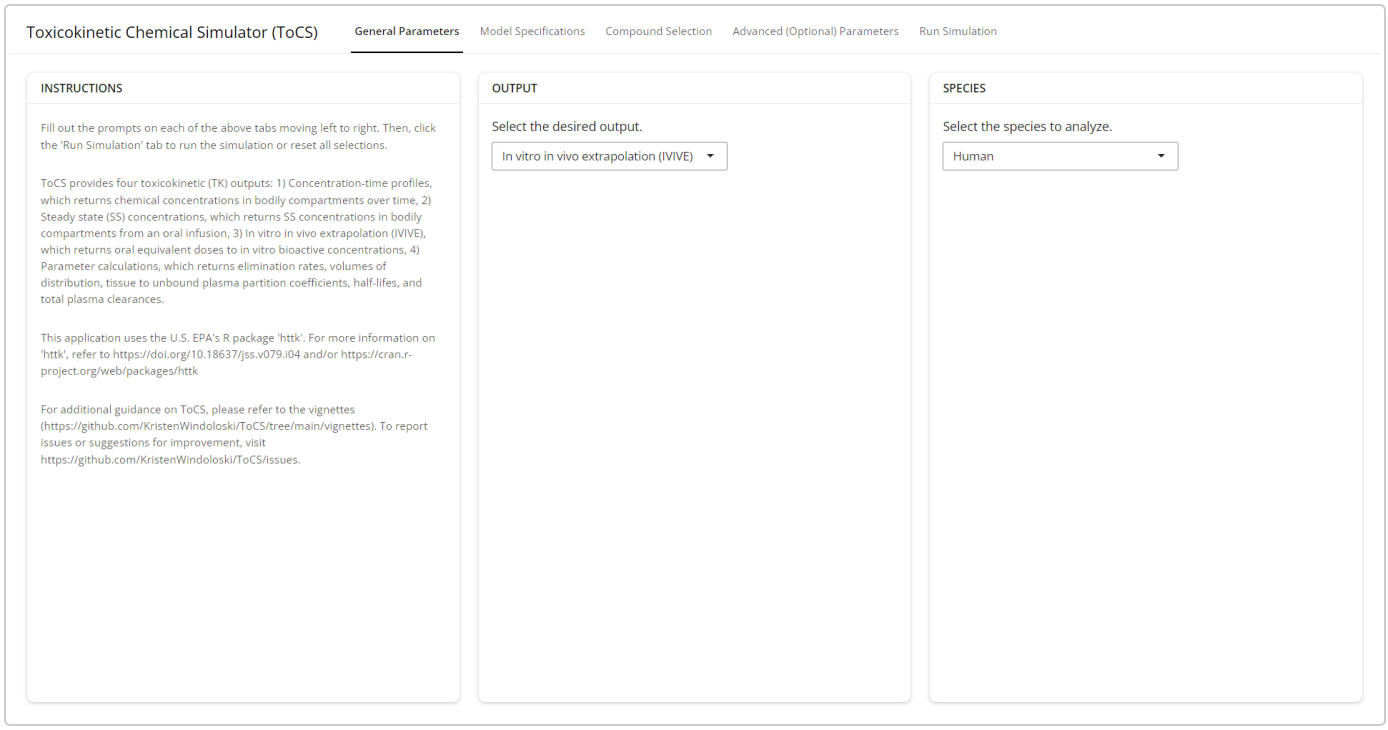
The opening interface to the ToCS app.

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.

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. Thus, the completed *General Parameters* tab should look like the image below.



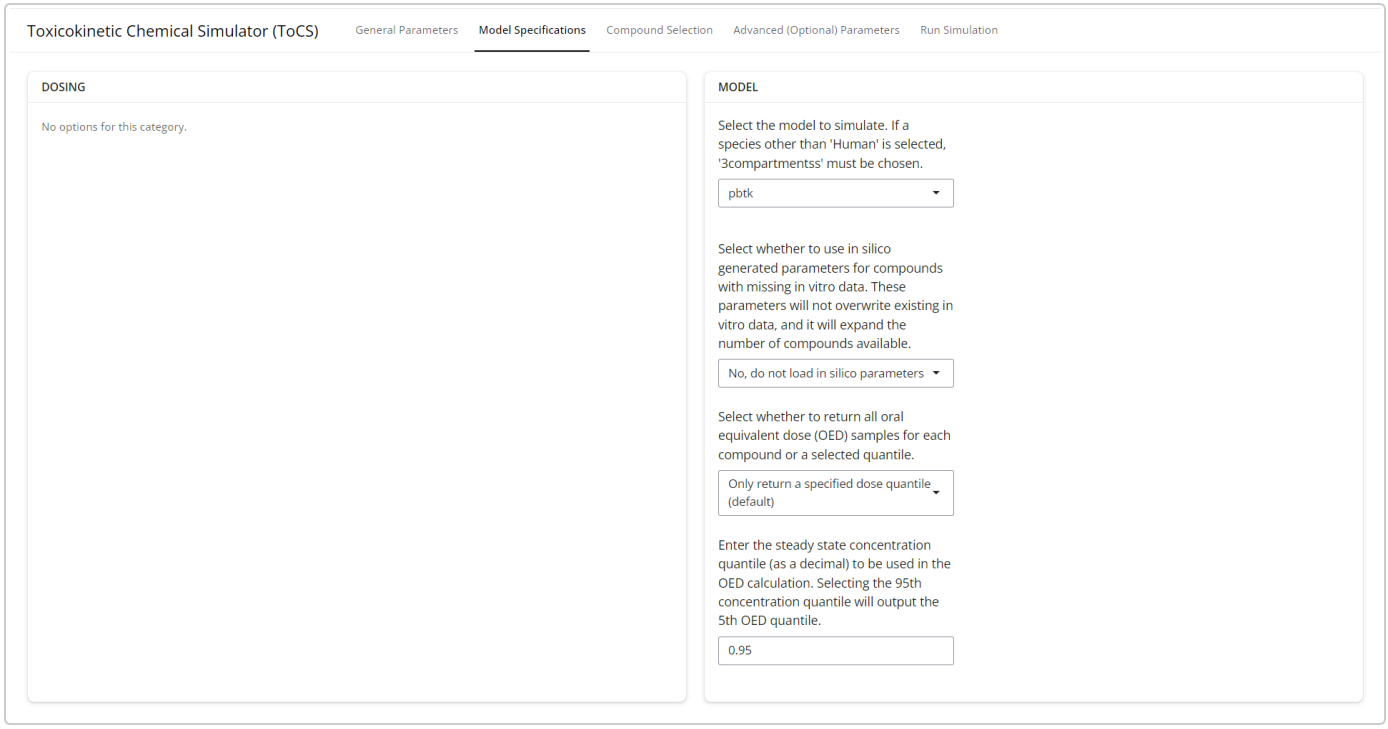
The completed General Parameters tab for example 1.

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.

Then, the third 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.



The completed model specifications tab for example 1 showing 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 httk 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. We then keep the second drop menu in the *Preloaded Compounds* card on *Choose from all available chemicals*. Then, the box below asks for the user to specify the fraction fetal bovine serum. We apply the assumption that it is 0.1 for the bioactivity assays for our desired 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 eight compounds that we would like to simulate and see that they are all present in the preloaded list. Thus, we select those compounds. On the *Uploaded Data* card on the right of the interface, there are three datasets that can be uploaded. The first one is if we need to upload any physical-chemical data for compounds we want to simulate that are not available under the preloaded compounds list. However, we do not need to upload anything here because all of the compounds we wanted were already available. The second spot to upload a file is required for IVIVE simulations. Here, we must upload a CSV file with bioactive concentrations (uM units) for each compound selected 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. All values were calculated as the 5th percentile of AC50s (active calls only) for each compound, where the list of AC50s was taken from the cHTS assay database from ICE.

ChemicalName	CAS	BioactiveConcentration
Acetamiprid	135410-20-7	7.35400
2,4-db	94-82-6	13.46000
Acephate	30560-19-1	0.05875
Acetochlor	34256-82-1	1.34800
Alachlor	15972-60-8	1.61700
Aldicarb	116-06-3	0.09300
Ametryn	834-12-8	1.08200
Amitraz	33089-61-1	27.49000

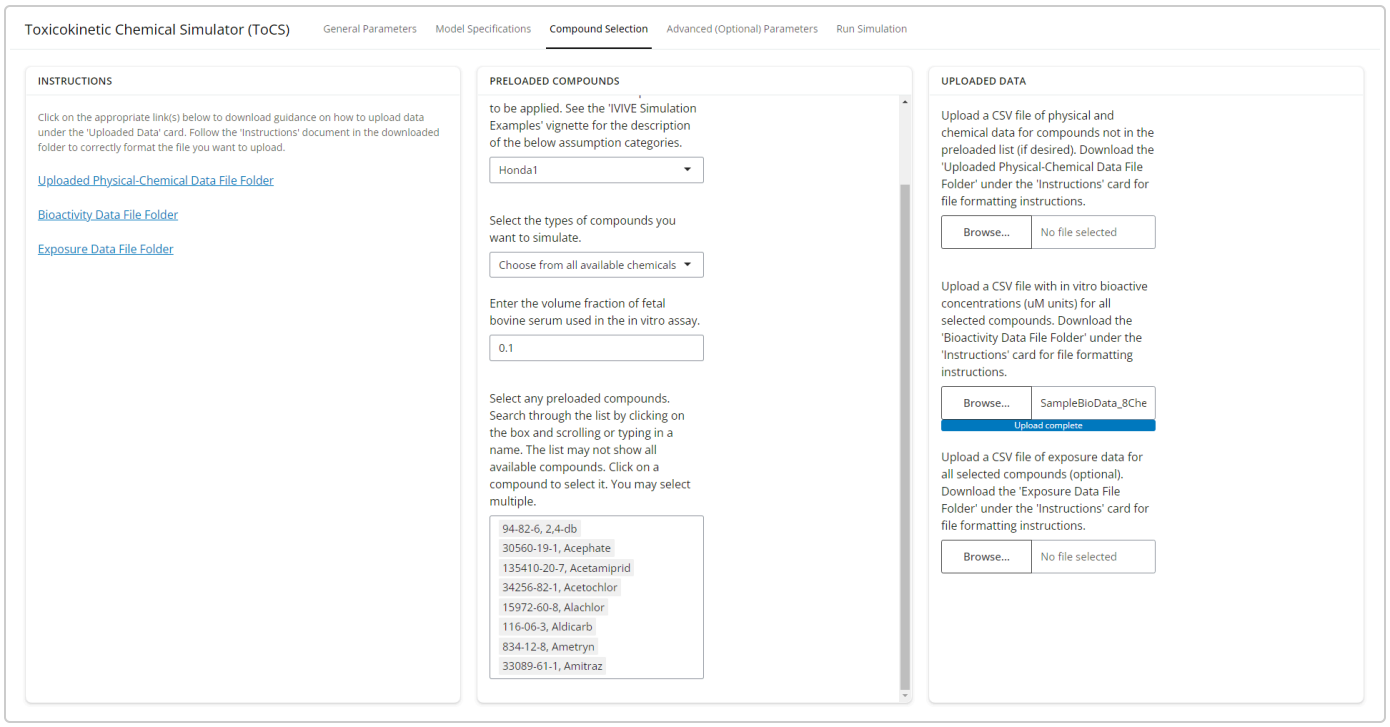
The third spot to upload a CSV file is for chemical exposure estimates, which is optional. Uploading this data will allow the user to visualize these exposure estimates against the model-outputted OEDs as well as a

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
Amitraz	33089-61-1	9.77e-04	1.24e-06	NA

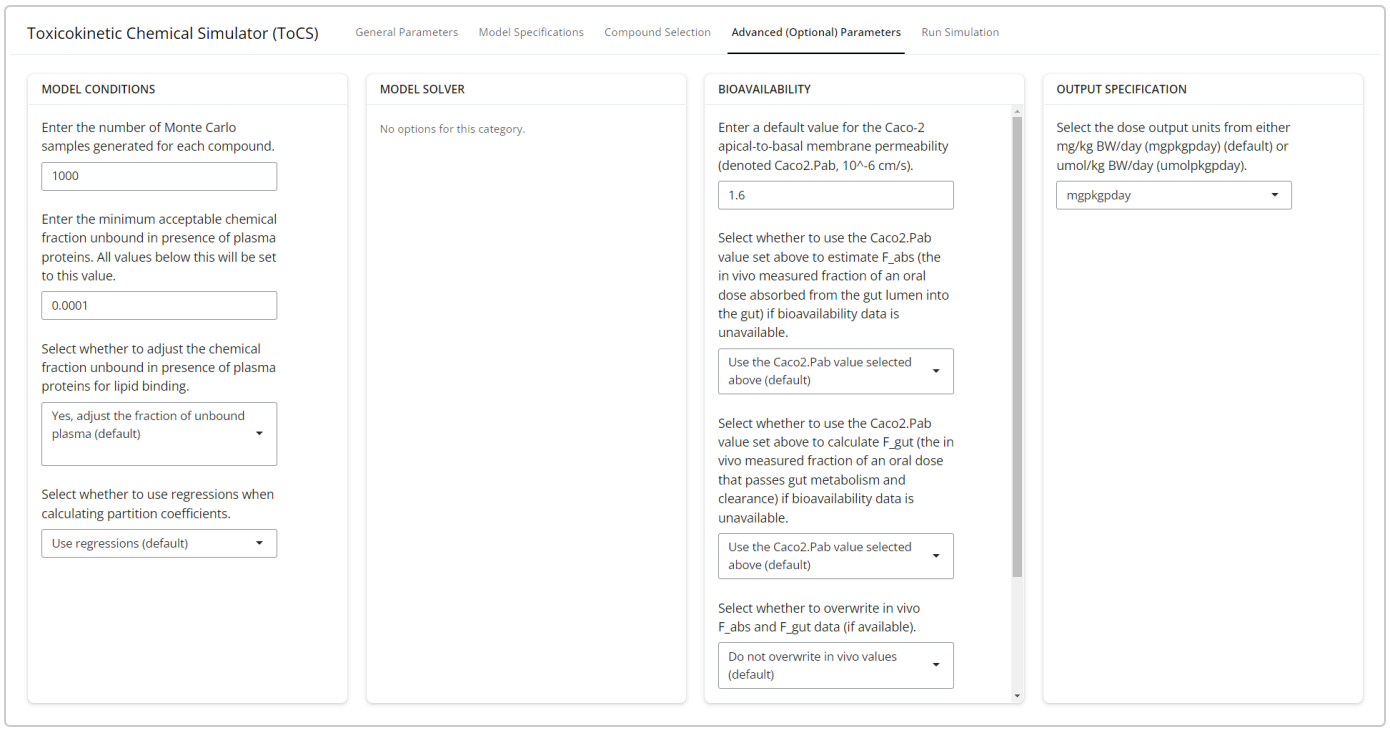
For help with exposure file formatting, please refer to the *Introduction to ToCS* vignette and/or the *Exposure Data File Folder* under the left-side *Instructions* card on the interface. Following the selection of the compounds to simulate as well as uploading bioactivity and exposure data files, the completed *Compound Selection* page should look like the image below.



The completed compound selection tab for example 1.

Advanced (Optional) Parameters Tab

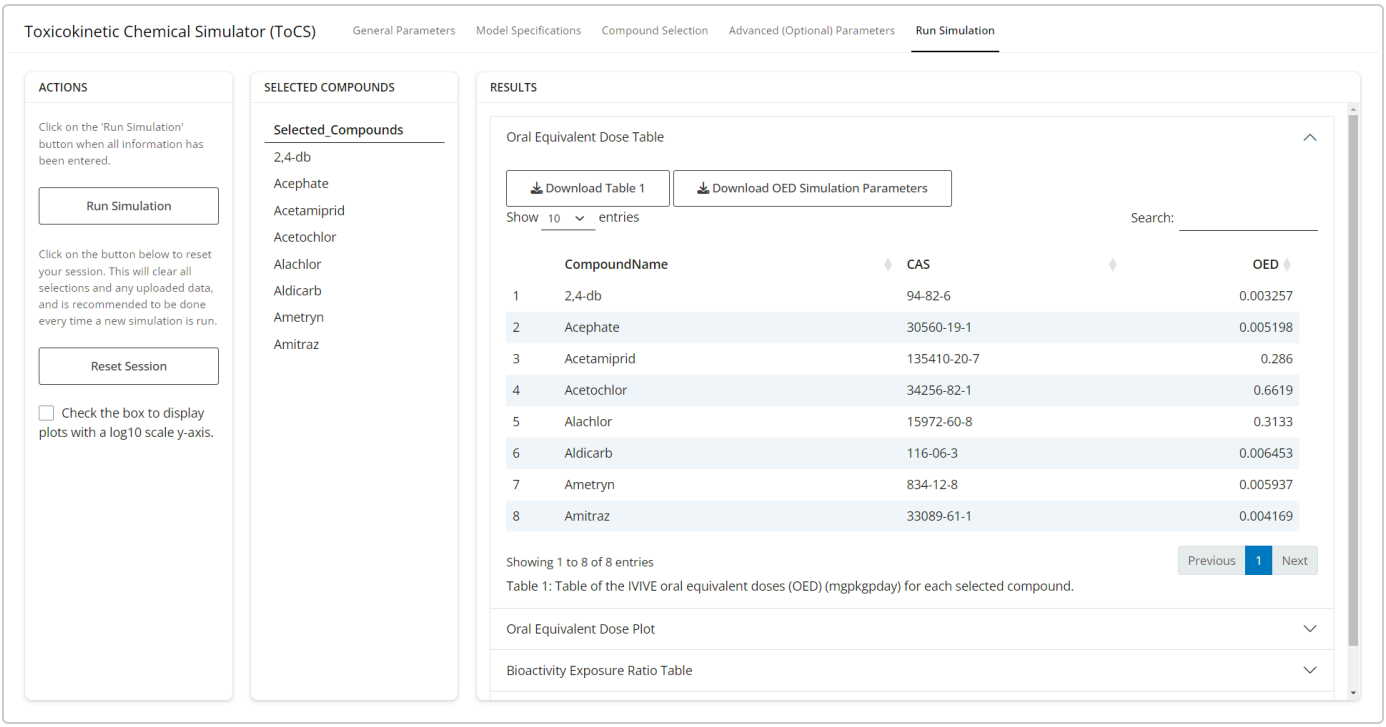
We will leave all options on this tab at their default values and proceed to the final *Run Simulation* tab. The *Advanced Parameters* tab should look like the image below.



The completed advanced parameters tab for example 1.

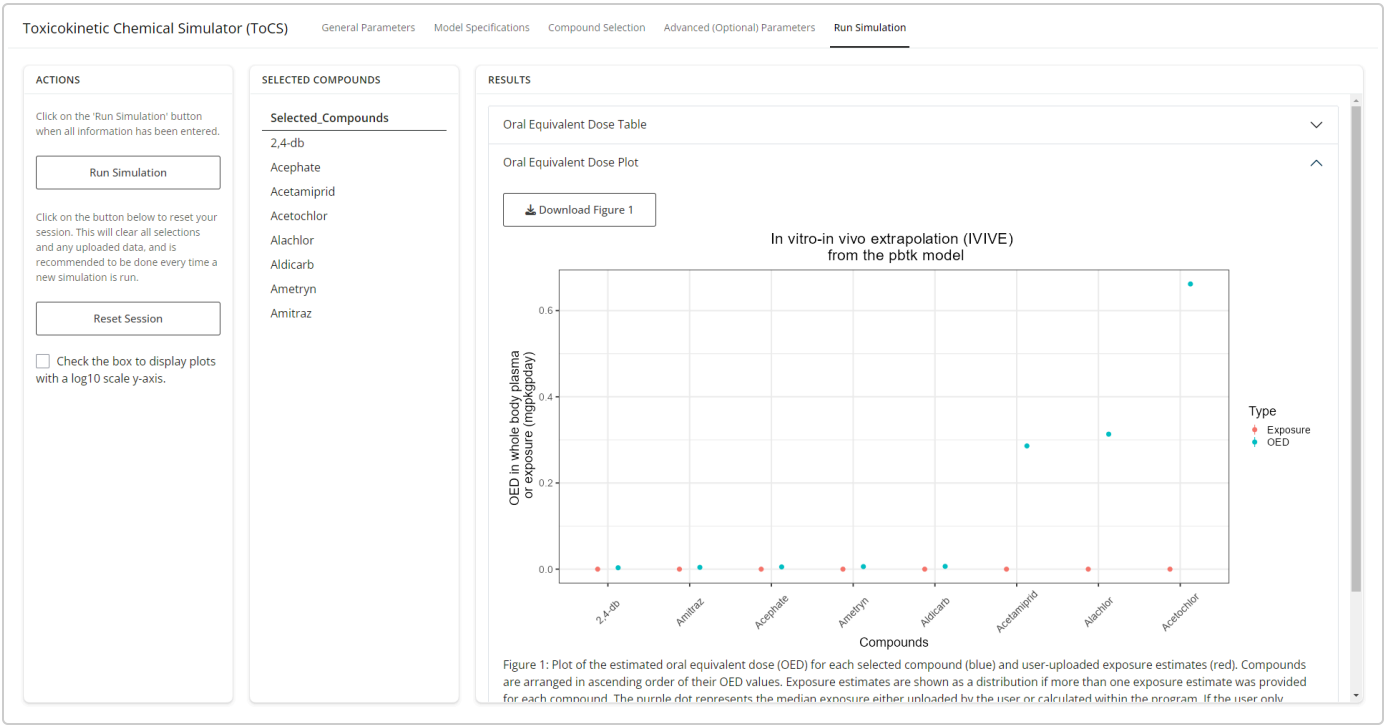
Run Simulation Tab

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.



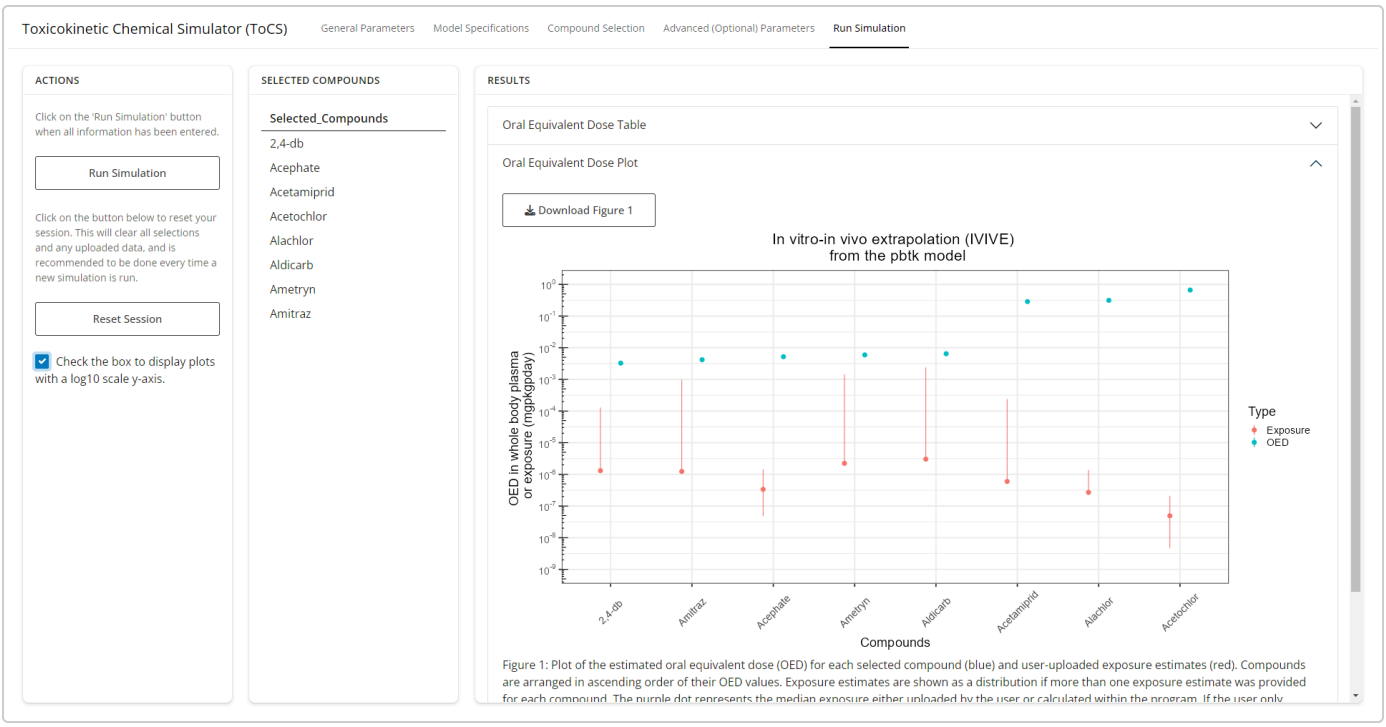
The completed run simulation tab for example 1 showing the expanded OED table tab.

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 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.



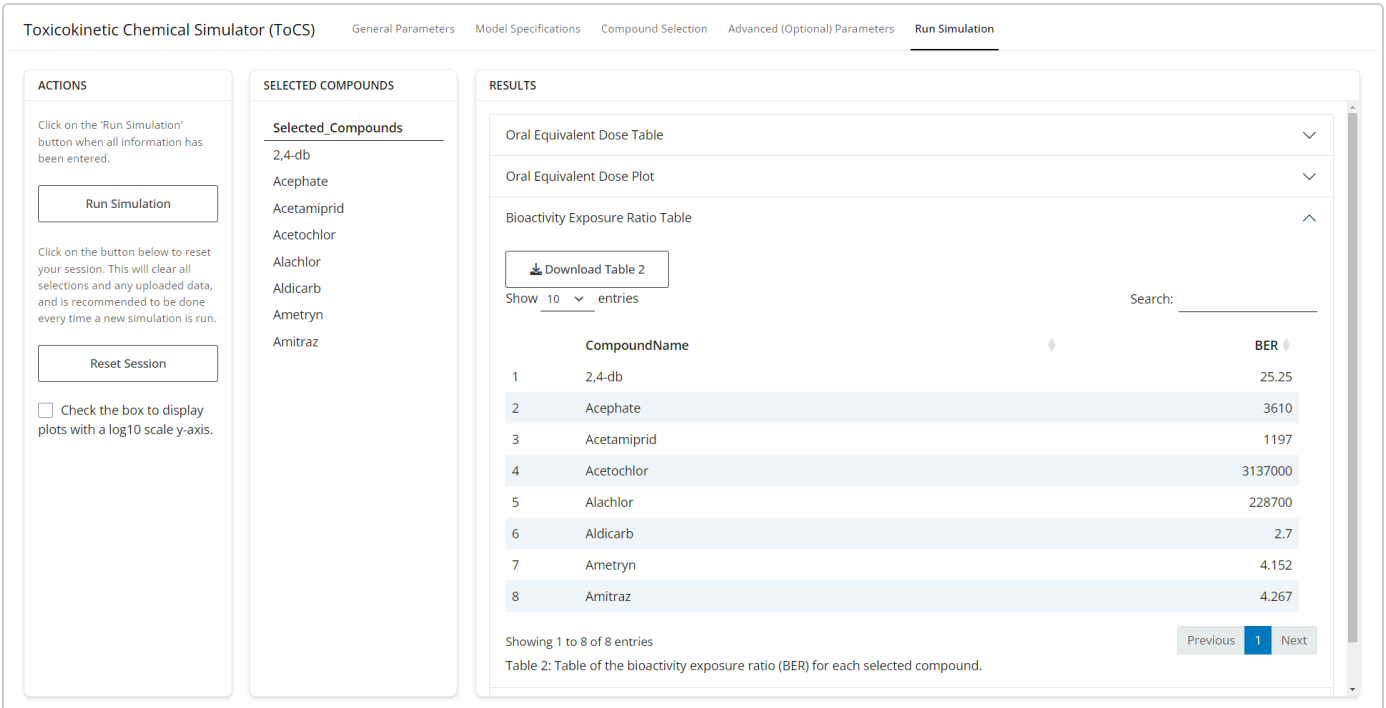
The completed run simulation tab for example 1 showing the expanded OED plot tab where the plot has a linear scale y-axis.

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.



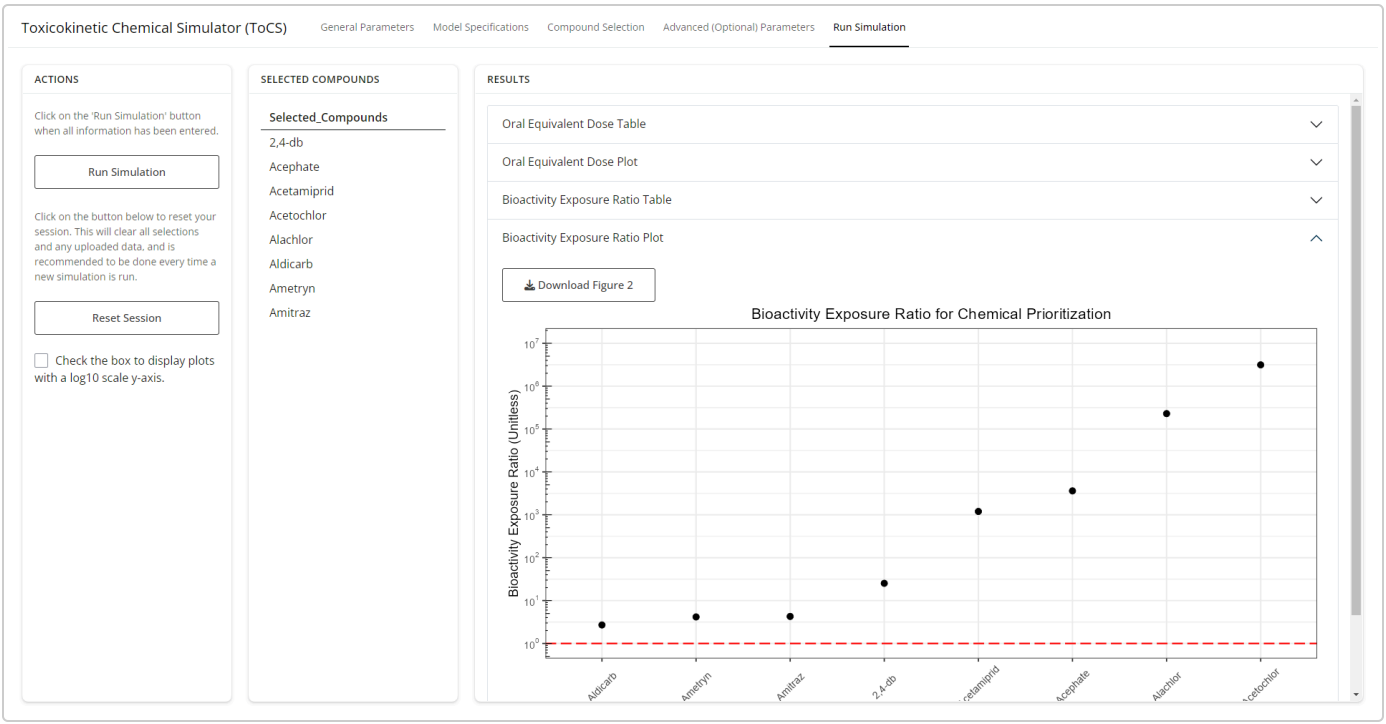
The completed run simulation tab for example 1 showing the expanded OED plot tab where the plot has a log10 scale y-axis.

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 further assessment. 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.

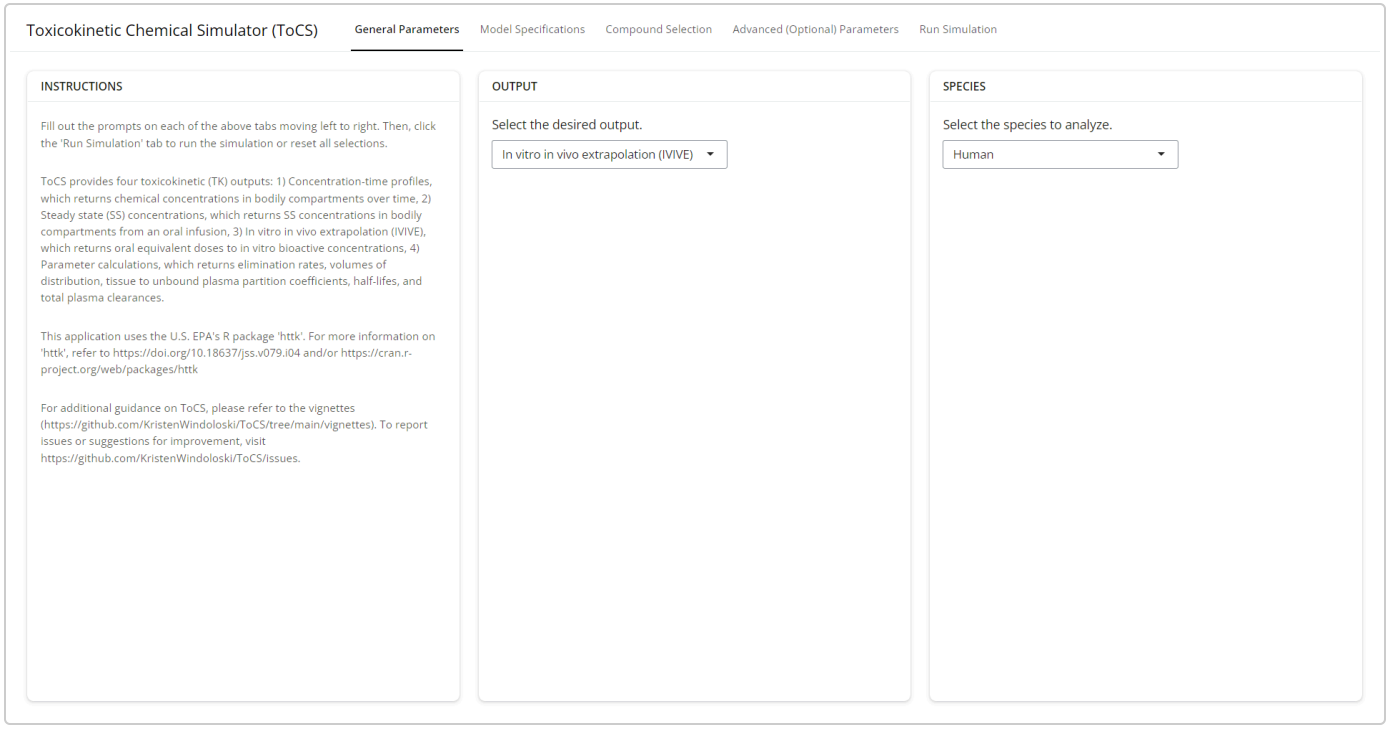
If we wanted to run another simulation, we would click the *Reset Session* button under the *Actions* button, which would clear all parameter inputs and simulations and return the interface to the *General Parameters* tab.

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

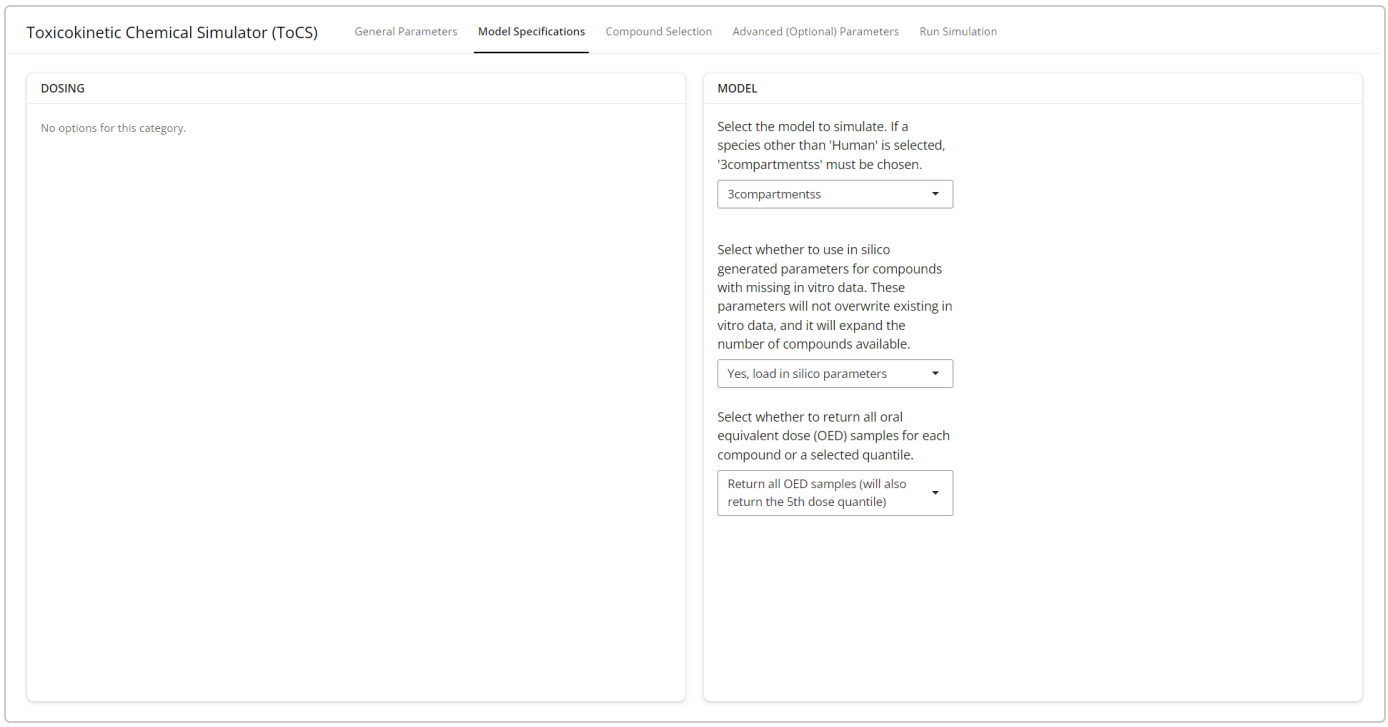
Since we want to perform IVIVE, we select the *In vitro in vivo extrapolation (IVIVE)* option under the drop down menu from the *Output* card. Then under the *Species* card, we select *Human* for the first drop down. Thus, the completed *General Parameters* tab should look like the image below.



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. Then, the third 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 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). We keep the second drop menu in the *Preloaded Compounds* card on *Choose from all available chemicals* and then the box below contains a list of preloaded compounds that we can select from. We search for the ten compounds we want to simulate and see that they are all present in the preloaded list. Thus, we select those compounds. Under the *Uploaded Data* card, we ignore the first file selection option since all compounds to simulate were found in the preloaded compounds list. Then for the second file selection option, we are required to upload a CSV file with bioactivity data in it for each selected compound. 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. All values were calculated as the 5th percentile of AC50s (active calls only) for each compound, where the list of AC50s was taken from the cHTS assay database from ICE. Abamectin did not have any AC50s, so one was entered to fill the data set.

ChemicalName	CAS	BioactiveConcentration
Acetamiprid	135410-20-7	7.35400

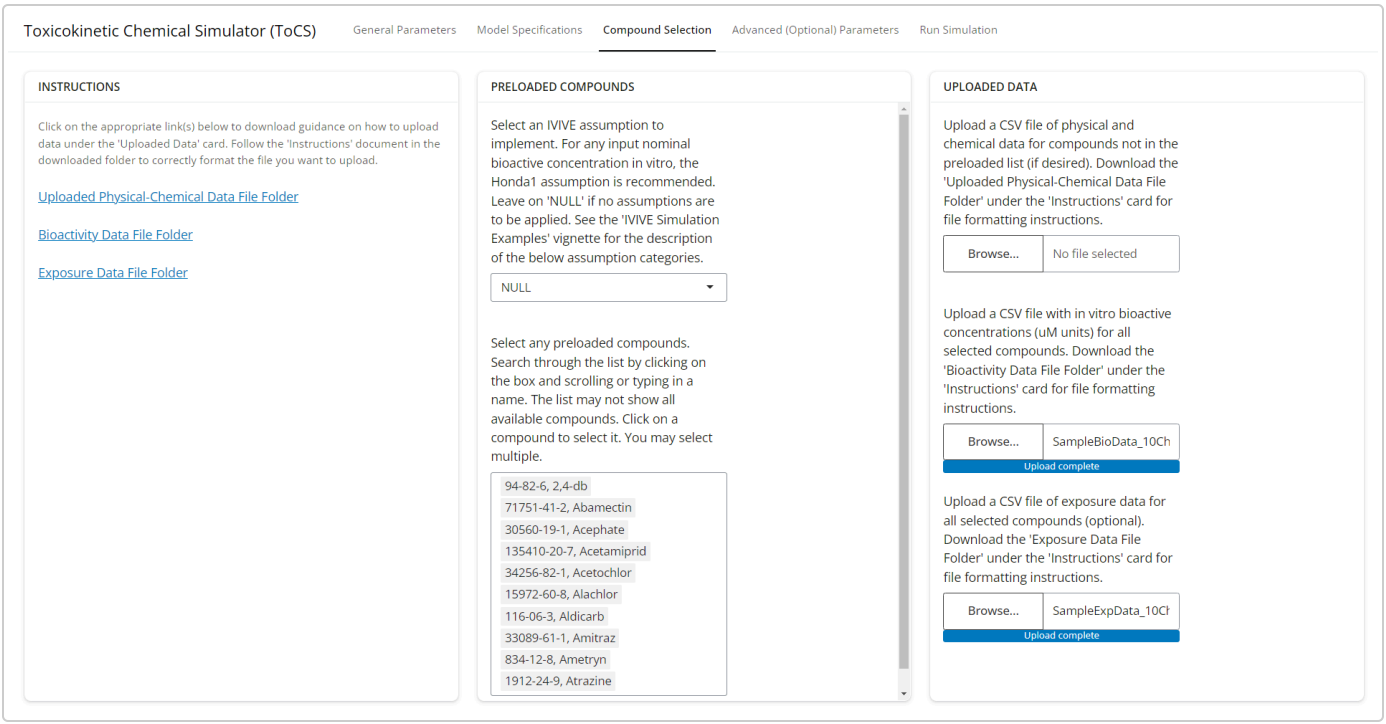
ChemicalName	CAS	BioactiveConcentration
2,4-db	94-82-6	13.46000
Acephate	30560-19-1	0.05875
Abamectin	71751-41-2	1.00000
Acetochlor	34256-82-1	1.34800
Alachlor	15972-60-8	1.61700
Aldicarb	116-06-3	0.09300
Ametryn	834-12-8	1.08200
Amitraz	33089-61-1	27.49000
Atrazine	1912-24-9	1.30200

For formatting instructions, please either download the *Bioactivity Data File Folder* on the left side of the page or consult the *Introduction to ToCS* vignette. The final file upload option under the *Uploaded Data* card provides the user the opportunity to upload chemical exposure data. Thus, we upload the following CSV file:

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
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

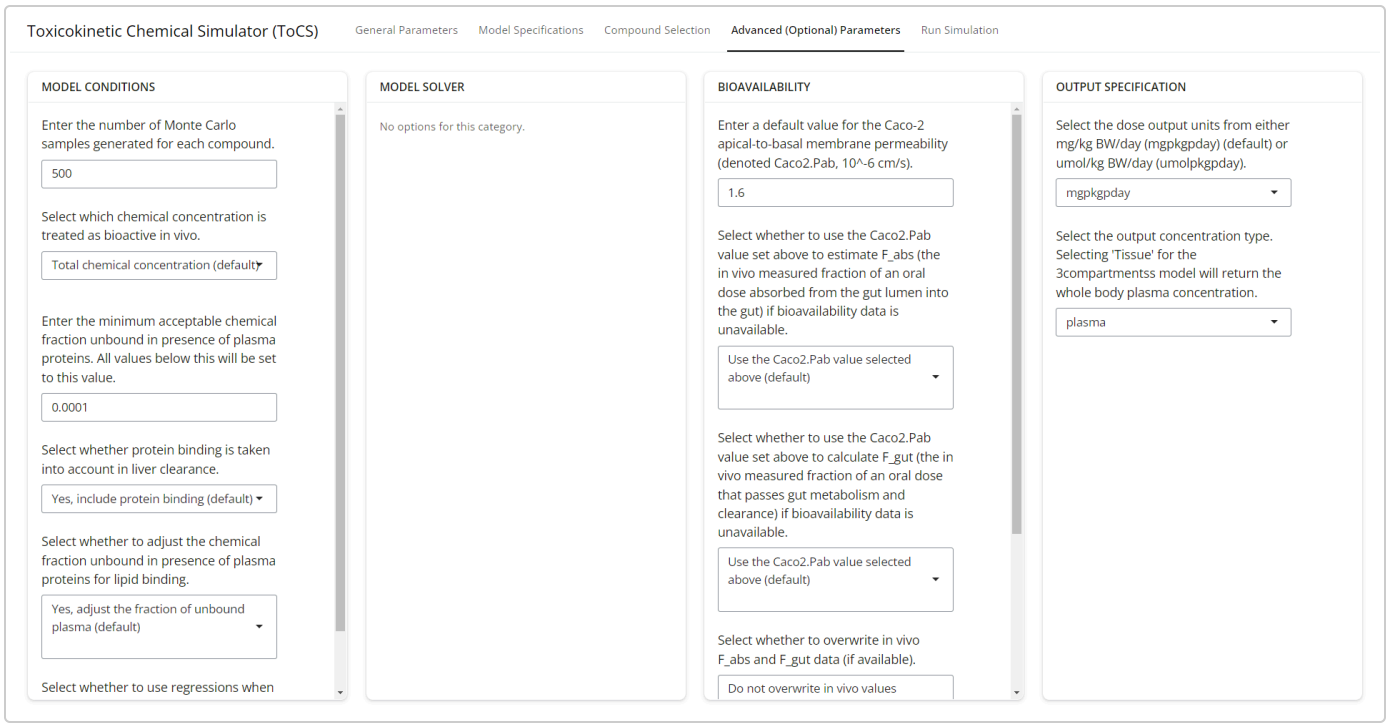
For more details on the format of this file, please review the *Introduction to ToCS* vignette and/or the Exposure Data File Folder below the upload area in the interface. The completed *Compound Selection* tab should look like the images below.



The completed compound selection tab for example 2.

Advanced (Optional) Parameters Tab

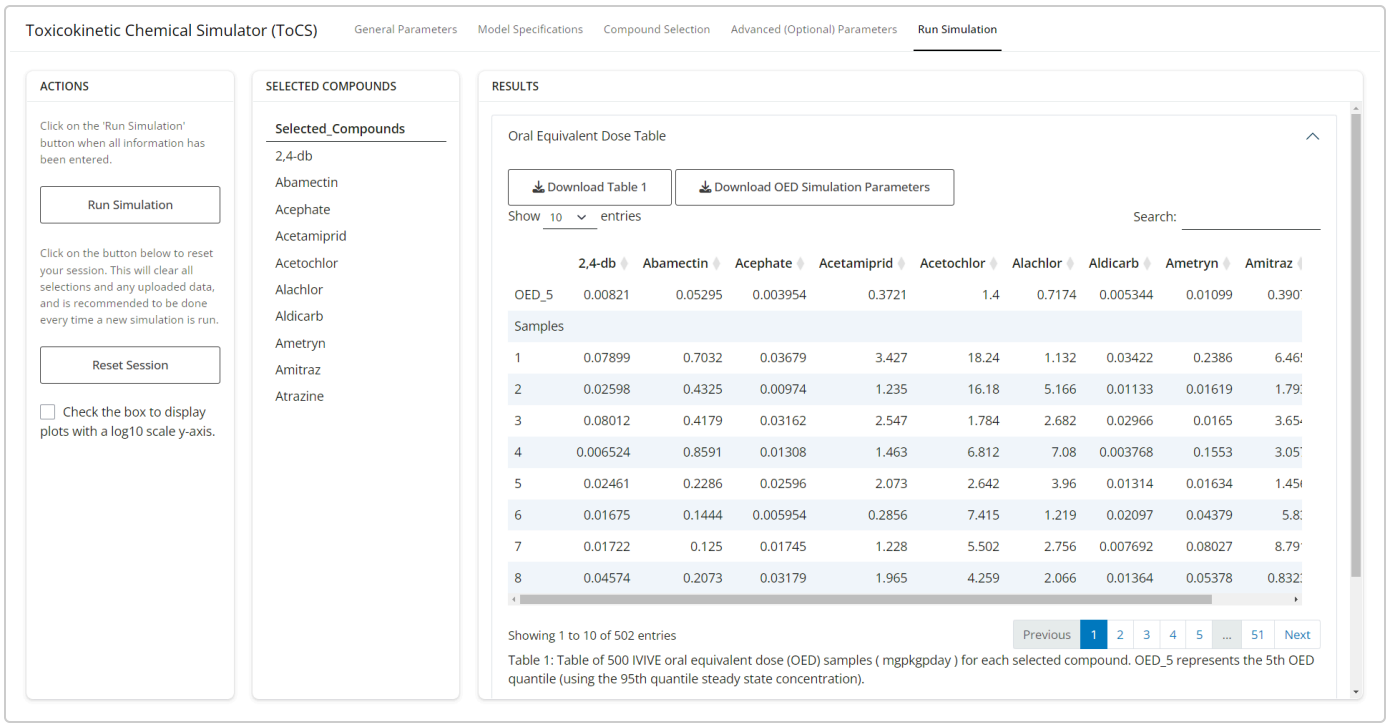
To speed up the computation time of the program, let's change the number of Monte Carlo samples generated for each compound (under the *Model Conditions* card) from 1000 to 500. Also, since we want to output the plasma OED, we keep the second drop down menu as *plasma*. Thus, the completed *Advanced Parameters* tab should look like the image below.



The completed advanced parameters tab for example 2.

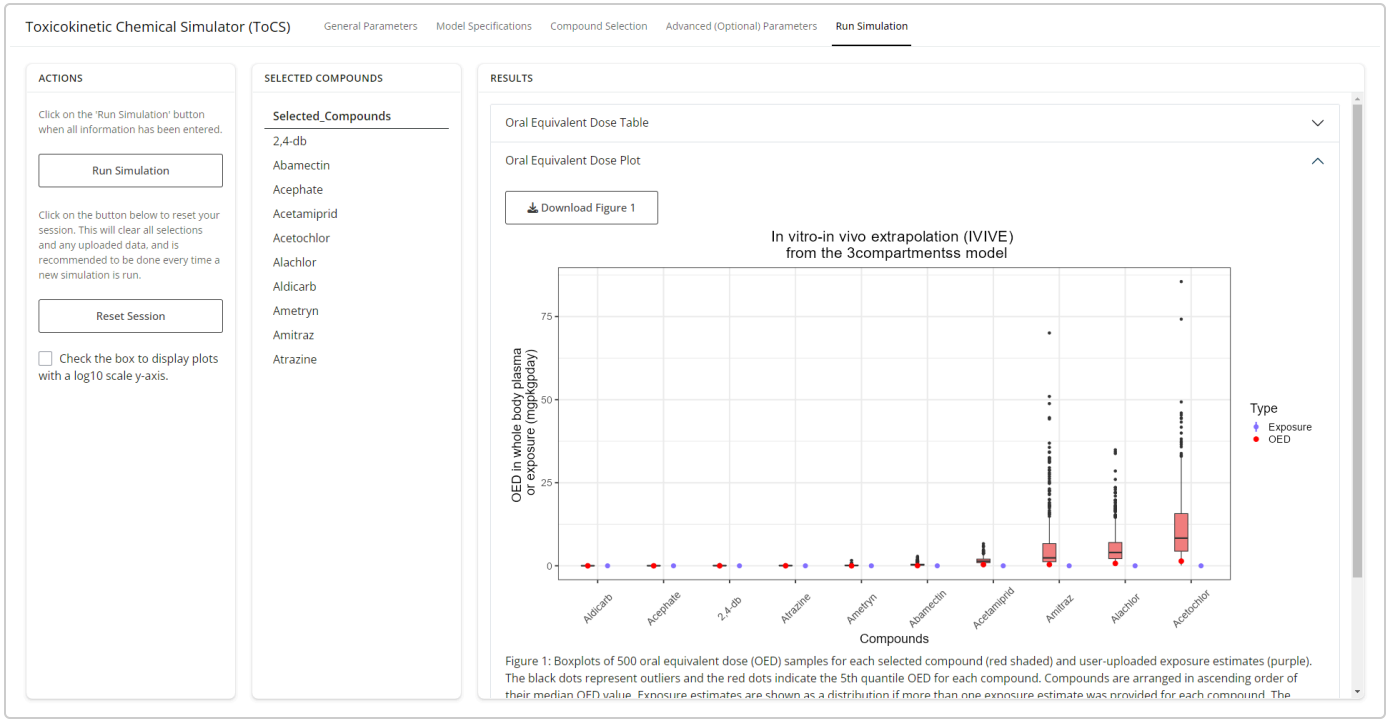
Run Simulation Tab

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.



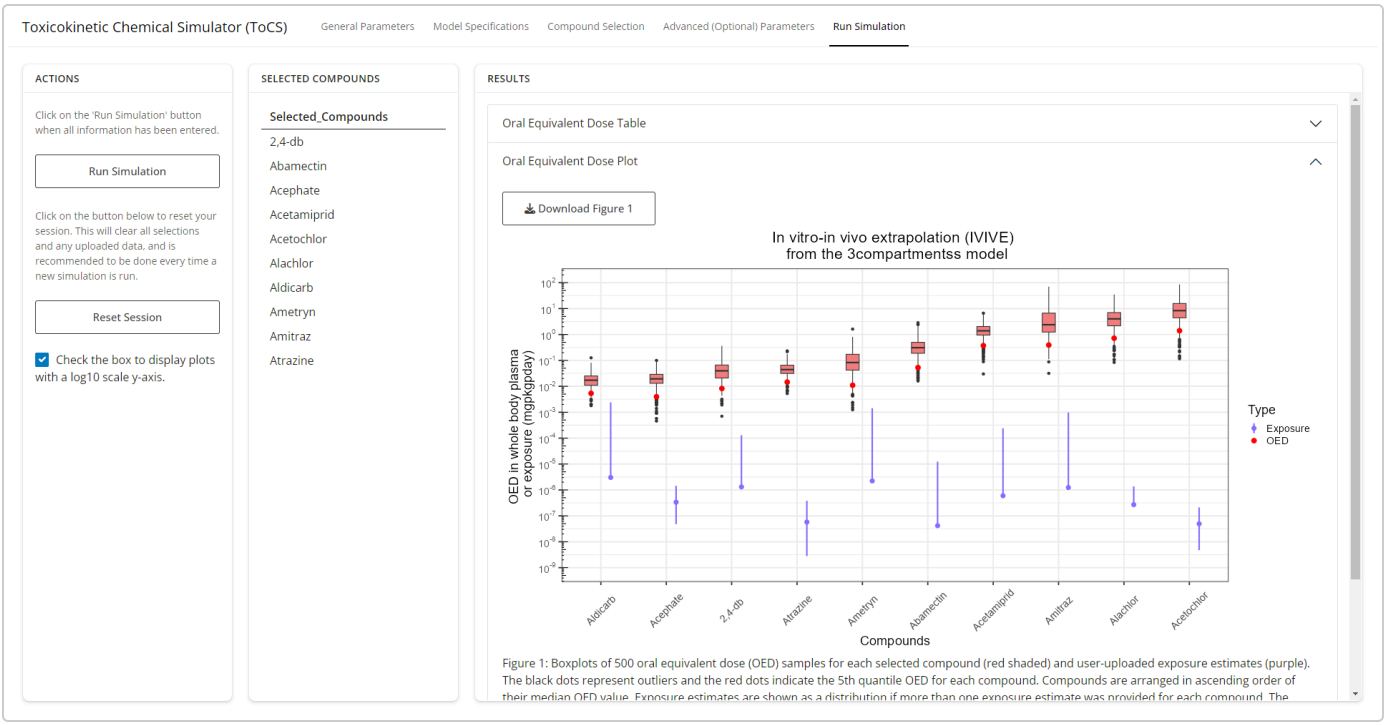
The completed run simulation tab for example 2 showing the expanded OED table tab.

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.



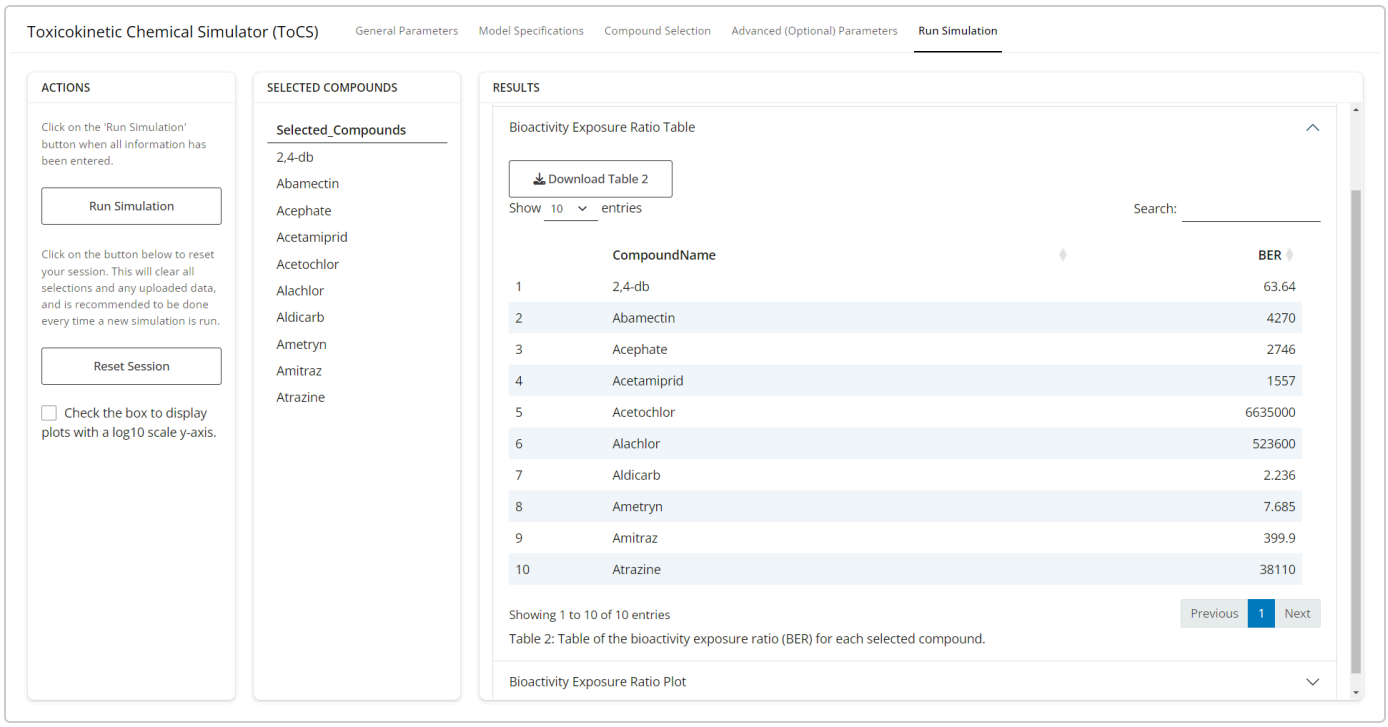
The completed run simulation tab for example 2 showing the expanded OED plot tab where the OED plot has a linear scale y-axis and showcases the OED distributions against the chemical exposure estimate distributions.

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.



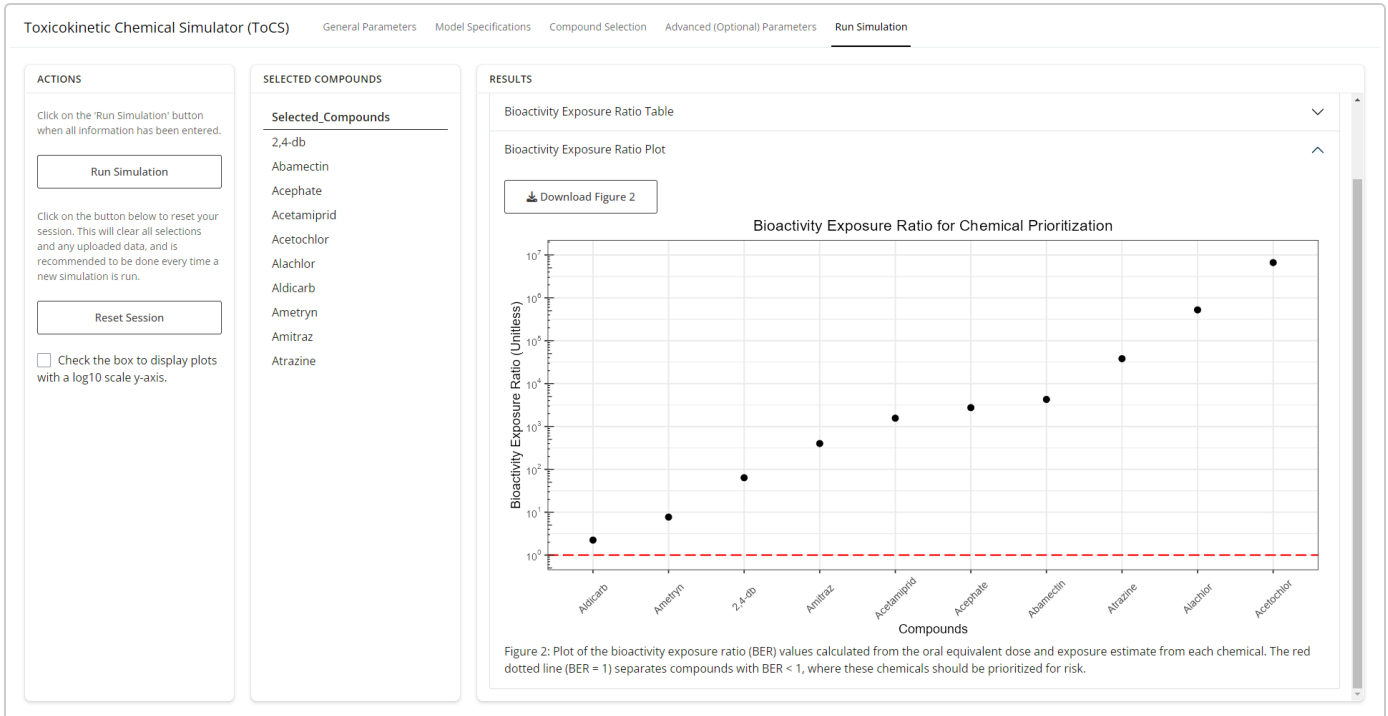
The completed run simulation tab for example 2 showing the expanded OED plot tab where the plot has a log10 scale y-axis.

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.



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 >> 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.

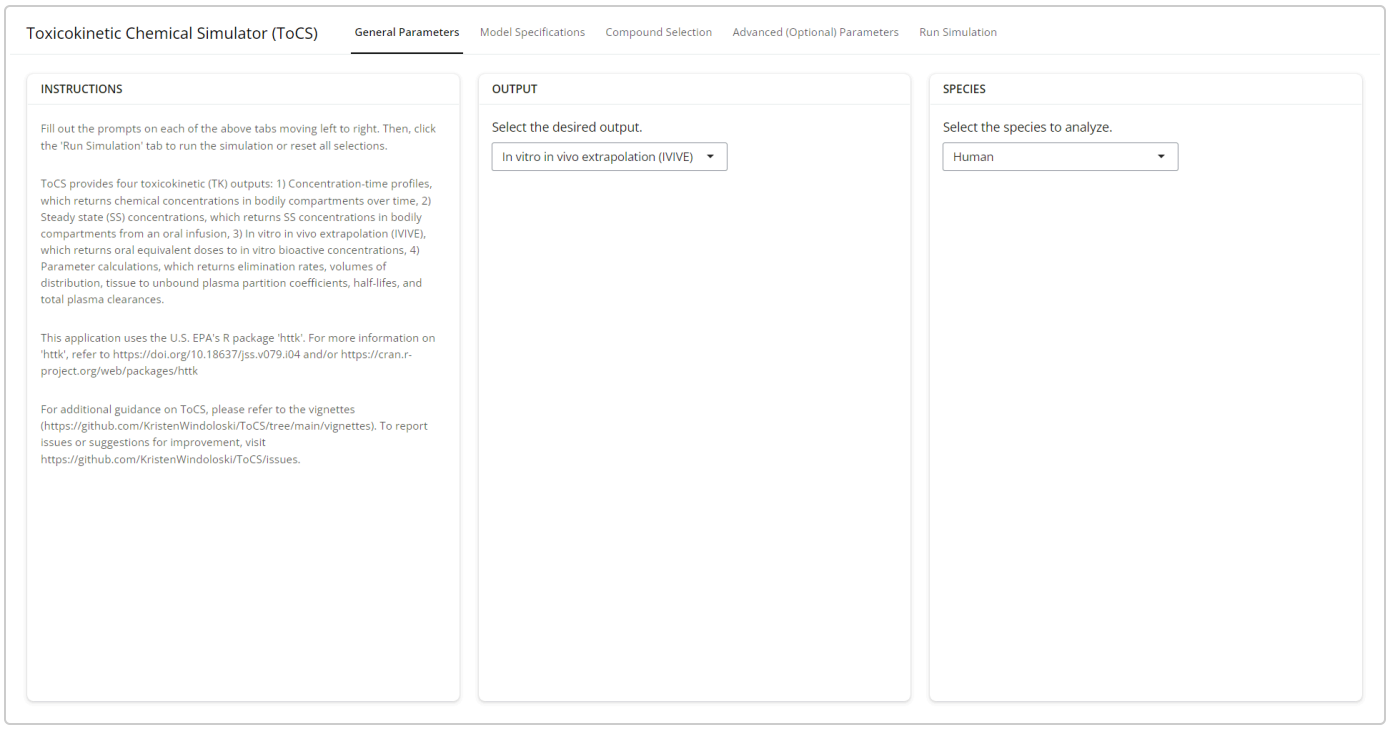
If we wanted to run another simulation, we would click the *Reset Session* button under the *Actions* button, which would clear all parameter inputs and simulations and return the interface to the *General Parameters* tab.

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. Thus, the completed *General Parameters* tab should look like the image below.

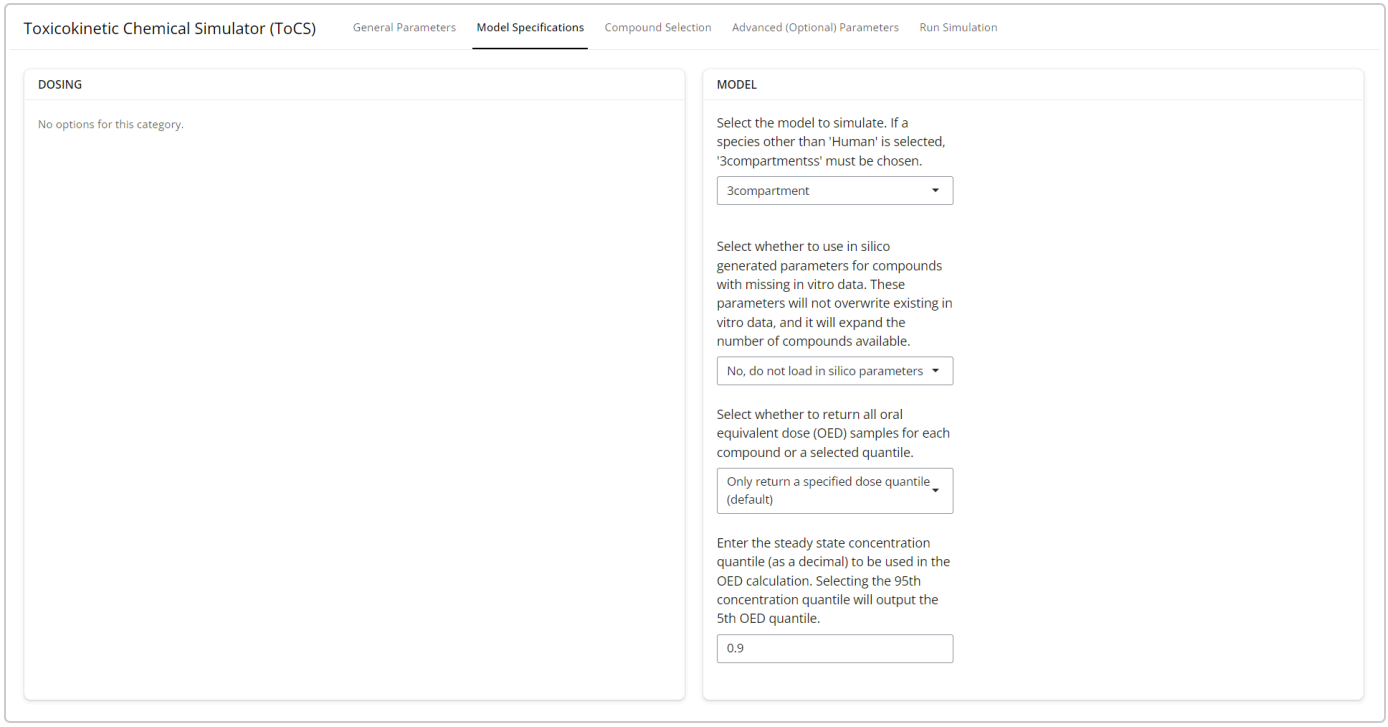


The completed General Parameters tab for example 3.

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. Then, the third 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.



The completed model specifications tab for example 3.

Compound Selection Tab

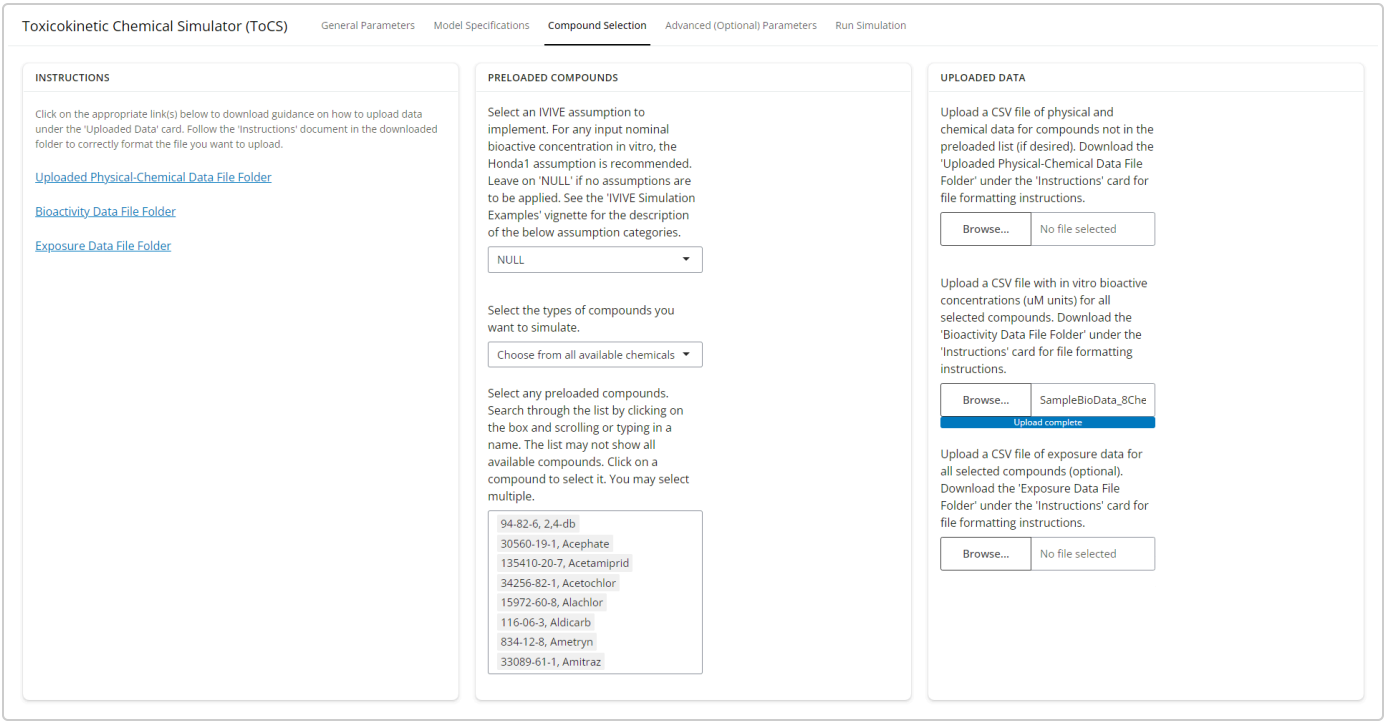
As with the first example, the first drop down menu under the *Preloaded Compounds* card has the user select a set of simulations assumptions to implement. See example 1 for descriptions of the assumptions. As with example 2, we will select NULL for the first drop down menu since we wanted to use 1) the nominal in vitro concentration as bioactive, 2) restrictive clearance, and 3) liver tissue as bioactive in vivo. We keep the second drop menu in the *Preloaded Compounds* card on *Choose from all available chemicals* and then in the box below that, there's a list of preloaded compounds that we can select from. We search for the eight compounds that we want to select and see that they are all present in the preloaded list. Thus, we select those compounds. Under the *Uploaded Data* card, we ignore the first file upload option since all of the compounds we want to simulate are available under the preloaded list in the middle column. The second file upload for bioactivity data is required, and so we upload the following csv table of bioactive concentrations for all compounds:

A csv file with bioactivity data for all eight chemicals. All values were calculated as the 5th percentile of AC50s (active calls only) for each compound, where the list of

AC50s was taken from the cHTS assay database from ICE.

ChemicalName	CAS	BioactiveConcentration
Acetamiprid	135410-20-7	7.35400
2,4-db	94-82-6	13.46000
Acephate	30560-19-1	0.05875
Acetochlor	34256-82-1	1.34800
Alachlor	15972-60-8	1.61700
Aldicarb	116-06-3	0.09300
Ametryn	834-12-8	1.08200
Amitraz	33089-61-1	27.49000

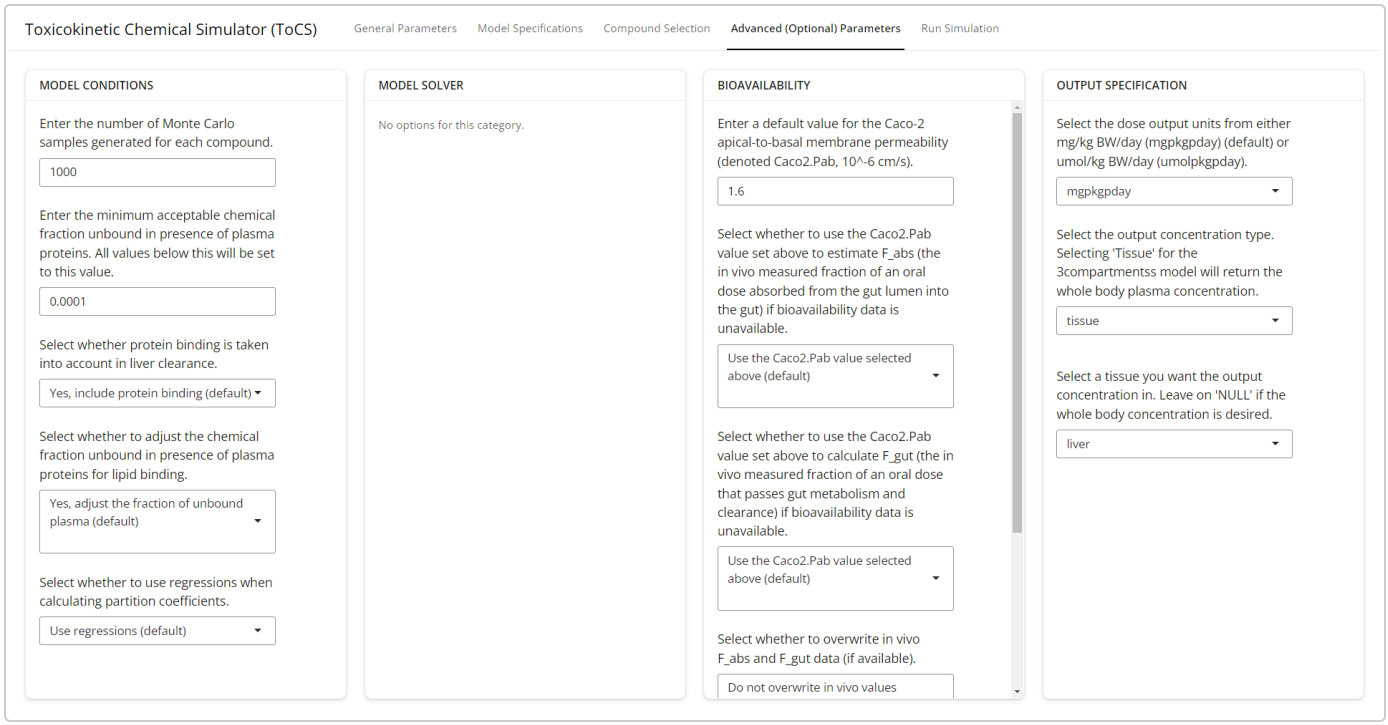
For formatting instructions, please either download the *Bioactivity Data File Folder* on the left side of the page or consult the *Introduction to ToCS* vignette. The final file upload option under the *Uploaded Data* card provides the user the opportunity to upload chemical exposure data. However, suppose that we do not have chemical exposure data to upload for this simulation. Therefore, we leave that blank. The completed *Compound Selection* tab should look like the images below.



The completed compound selection tab for example 3.

Advanced (Optional) Parameters Tab

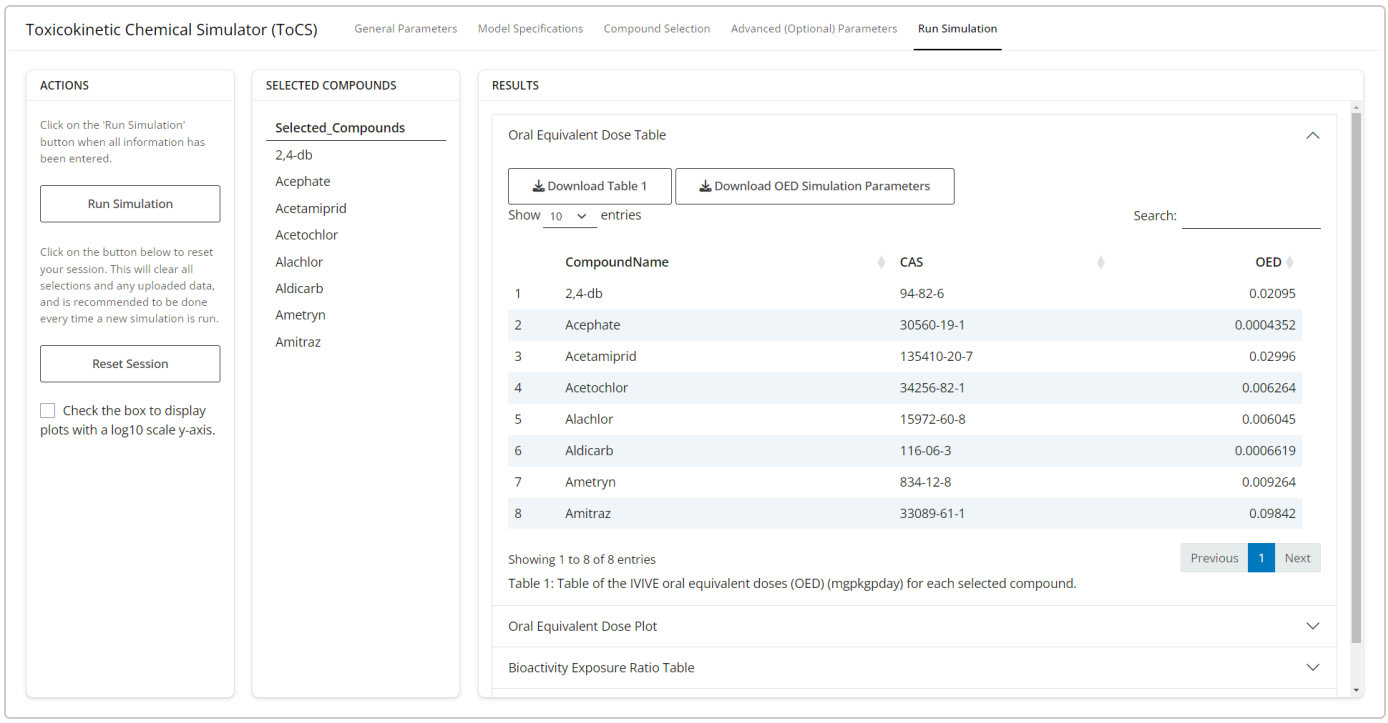
Since we want to output the OED in the liver, we select *tissue* under the second drop down menu in the *Output Specification* card specifying the output concentration type. This prompts the appearance of a third drop down menu. We select *liver* from this menu since we want the liver OED. No changes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



The completed advanced parameters tab for example 3.

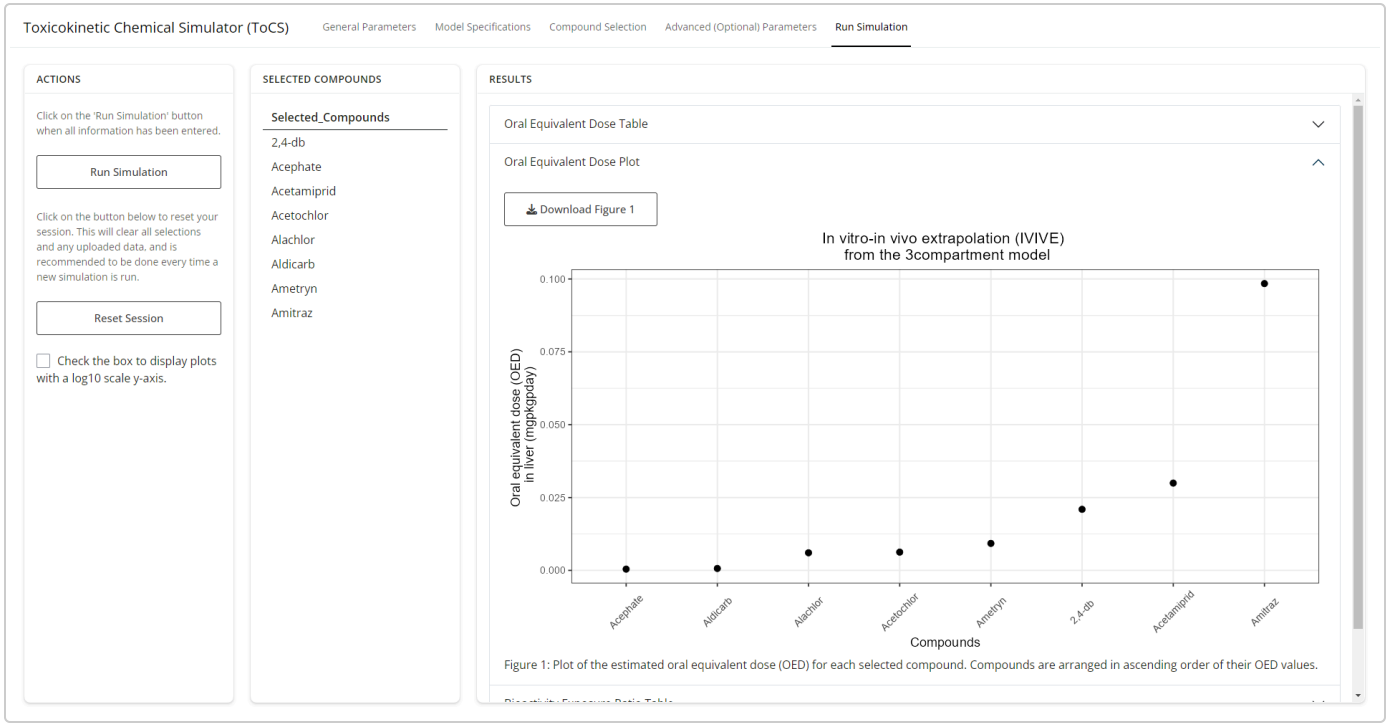
Run Simulation Tab

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.



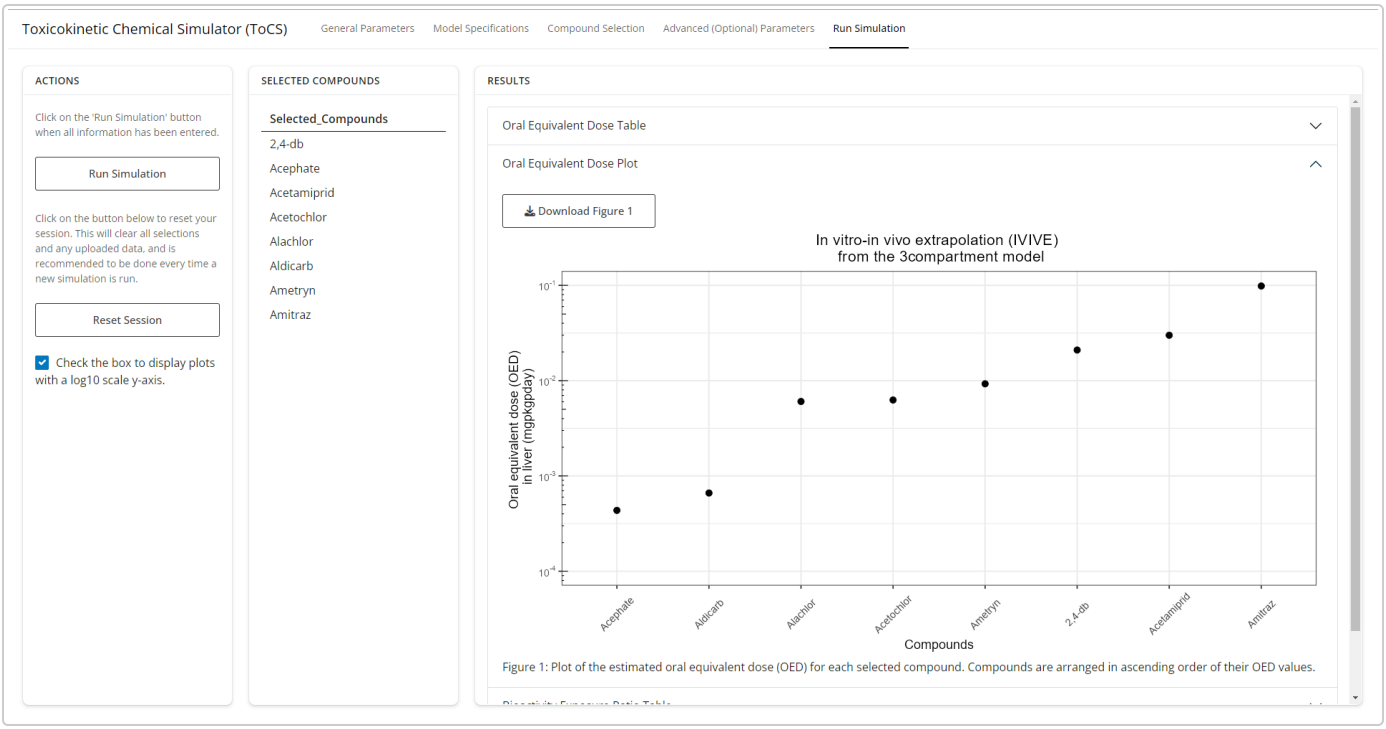
The completed run simulation tab for example 3 showing the expanded OED table tab.

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.



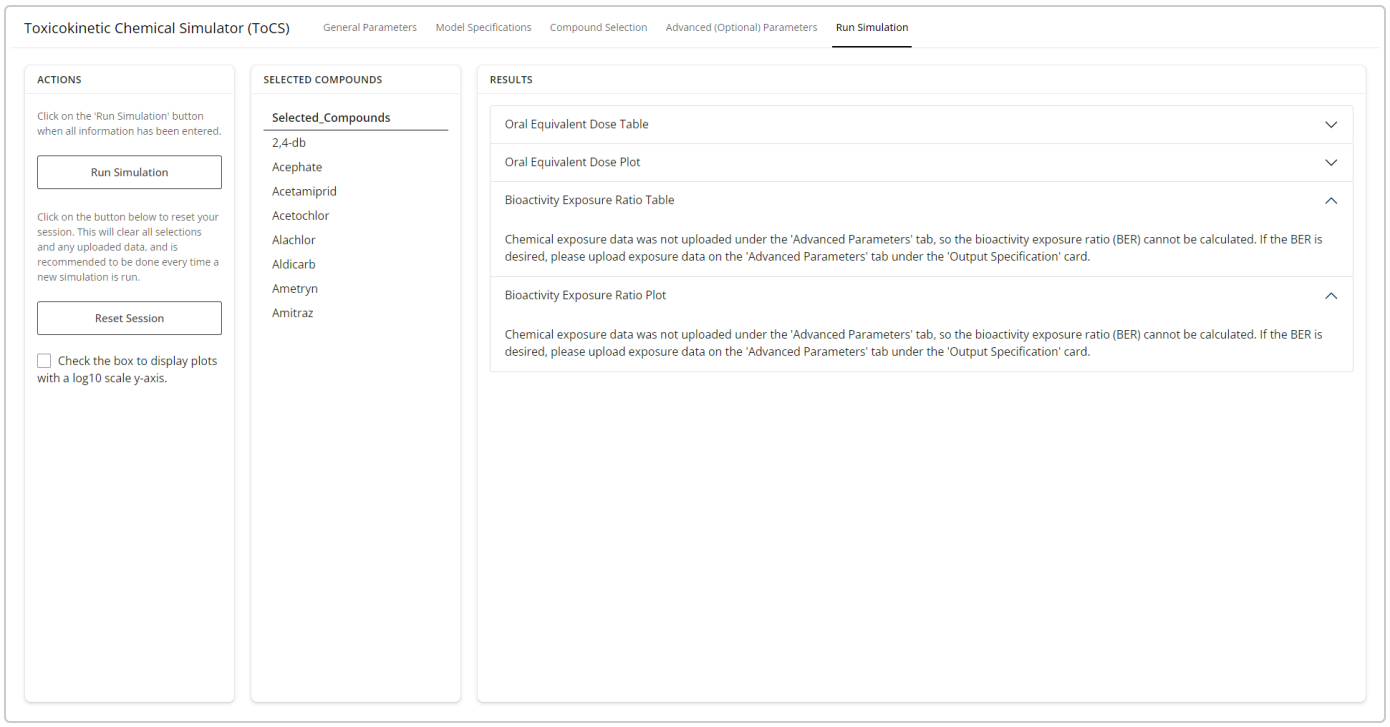
The completed run simulation tab for example 3 showing the expanded OED plot tab where the plot has a linear scale y-axis.

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.



The completed run simulation tab for example 3 showing the expanded BER table and plot tabs, which notifies the user that the calculations were not computed due to lack of exposure data.

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.