

# Steady State Concentration 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 ToCS README file 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 two examples that use the ToCS app to generate steady state concentrations, 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 initial interface of the ToCS app. At the top, there is a navigation bar with tabs: General Parameters (which is active), Model Specifications, Compound Selection, Advanced (Optional) Parameters, and Run Simulation. Below the navigation bar are three main input sections:

- INSTRUCTIONS:** A section containing instructions and a note about outputs. It says: "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 notes: "ToCS provides four outputs: 1) Concentration-time profiles (returns chemical concentrations in body compartments over time), 2) Steady state (SS) concentration (returns SS concentrations in body compartments from an oral infusion), 3) In vitro to in vivo extrapolation (IVIVE) (returns oral equivalent doses to in vitro bioactive concentrations), 4) Parameter calculations (returns elimination rates, volumes of distribution, tissue to unbound plasma partition coefficients, half-lives, and total plasma clearances)."
- OUTPUT:** A section titled "Select the desired output." with a dropdown menu set to "Select". A note below the dropdown says: "Must not be equal to Select."
- SPECIES:** A section titled "Select the species to analyze." with a dropdown menu set to "Select". A note below the dropdown says: "Must not be equal to Select."

The opening interface to the ToCS app for example 1.

# Example 1

In this example, let's say we want to generate human steady state concentrations in whole body plasma. We want to use a constant daily dose of 1 mg/kg BW with the PBTK model and make compounds with only in silico generated parameters (hepatic clearance, fraction unbound in plasma) also available for selection. We do not have specific chemicals in mind, so we will just select chemicals from the preloaded list of chemicals.

## General Parameters Tab

Under the *Output* card, we select *Steady state concentrations* since that is the type of simulation we want to run. Under the *Species* card, we select *Human* for the first drop down menu. The completed *General Parameters* card is shown in the image below.

The screenshot shows the 'General Parameters' tab of the ToCS application. The top navigation bar includes links for 'General Parameters' (which is active), 'Model Specifications', 'Compound Selection', 'Advanced (Optional) Parameters', and 'Run Simulation'. The main interface is divided into three sections: 'INSTRUCTIONS', 'OUTPUT', and 'SPECIES'.

- INSTRUCTIONS:** A text area containing instructions for using the simulator, mentioning four output types: concentration-time profiles, steady state concentrations, in vitro to in vivo extrapolation (IVIVE), and parameter calculations. It also notes the use of the R package 'httk'.
- OUTPUT:** A section titled 'Select the desired output.' with a dropdown menu set to 'Steady state concentrations'.
- SPECIES:** A section titled 'Select the species to analyze.' with a dropdown menu set to 'Human'.

Below the main sections, there are several hyperlinks for additional resources: 'Vignettes (ToCS tutorials)', 'Report ToCS issues/suggestions', 'httk publication', and 'httk CRAN webpage'.

The completed General Parameters tab for example 1.

## Model Specifications Tab

Under the *Dosing* card, we leave the total daily dose as its default value of 1 mg/kg BW. Under the *Model* card, we select *pbtk* for the first drop down to use the pbtk model. For the second drop down, we select *Yes* since we want to include compounds with only in silico generated parameters into the selection availability on the next page. Thus, the completed model specifications tab should look like the image below.

The completed model specifications tab for example 1.

## Compound Selection Tab

Since we selected to load in silico generated parameters, the *Preloaded Compounds* card will take a few moments to load. Once loaded, keep the first drop menu in the *Preloaded Compounds* card on *Choose from all available chemicals* and then search through the list and select 10 compounds:

- Abamectin (CAS: 71751-41-2)
- Aldicarb (CAS: 116-06-3)
- Captan (CAS: 133-06-2)
- Fenarimol (CAS: 60168-88-9)
- Hexanedioic acid (CAS: 124-04-9)
- Isoborneol (CAS: 124-76-5)
- Pyrene (CAS: 129-00-0)
- Sodium Cyclamate (CAS: 139-05-9)
- Thiabendazole (CAS: 148-79-8)
- Tribufos (CAS: 78-48-8)

We leave the *Uploaded Data* card as is, and the completed *Compound Selection* tab should look like the image below.

Note that if we selected *No* on the previous *Model Specifications* tab for using in silico generated parameters then captan, hexanedioic acid, isoborneol, and sodium cyclamate would not be available for simulation under the *Preloaded Compounds* card (i.e. these compounds use in silico generated parameters instead of human in vitro data).

**INSTRUCTIONS**

Click on the appropriate link(s) below to download guidance on how to upload data under the 'Uploaded Data' card. Follow the 'Instructions' document in the downloaded folder to correctly format the file you want to upload.

[Uploaded Physical-Chemical Data File Folder](#)

**PRELOADED COMPOUNDS**

Select the types of compounds you want to simulate.

Choose from all available chemicals ▾

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.

71751-41-2, Abamectin | 116-06-3, Aldicarb | 133-06-2, Captan  
60168-88-9, Fenarimol | 124-04-9, Hexanedioic acid | 124-76-5, Isoborneol  
129-00-0, Pyrene | 139-05-9, Sodium cyclamate | 148-79-8, Thilabendazole  
78-48-8, Tribufos

**UPLOADED DATA**

Upload a CSV file of physical and chemical data for compounds not in the preloaded list (if desired). Download the 'Uploaded Physical-Chemical Data File Folder' under the 'Instructions' card for file formatting instructions.

Browse... No file selected

The completed compound selection tab for example 1.

## Advanced (Optional) Parameters Tab

Since we want to output steady state concentrations for whole body plasma, we leave the selections under the *Output Specification* card as their default values. We also leave the remaining three cards as their default values and proceed to the final tab. The *Advanced Parameters* tab should look like the image below.

<b>MODEL CONDITIONS</b>	<b>MODEL SOLVER</b>	<b>BIOAVAILABILITY</b>	<b>OUTPUT SPECIFICATION</b>
Select whether protein binding is taken into account in liver clearance.  Yes, include protein binding (default)	No options for this category.	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 the output concentration units.  uM  Select the output concentration type.  plasma  Select a tissue you want the output concentration in. Leave on 'NULL' if the whole body concentration is desired.  NULL
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 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 use regressions when calculating partition coefficients.  Use regressions (default)		Select whether to overwrite in vivo F_abs and F_gut data (if available).  Do not overwrite in vivo values (default)	
Enter the p-value threshold for the in vitro intrinsic hepatic clearance rate where clearance assay results with p-values above this threshold are set to zero.  0.05		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)	
Enter the minimum acceptable chemical fraction unbound in presence of plasma proteins. All values below this will be set to this value.  0.0001			

The edited Advanced Parameters tab for example 1.

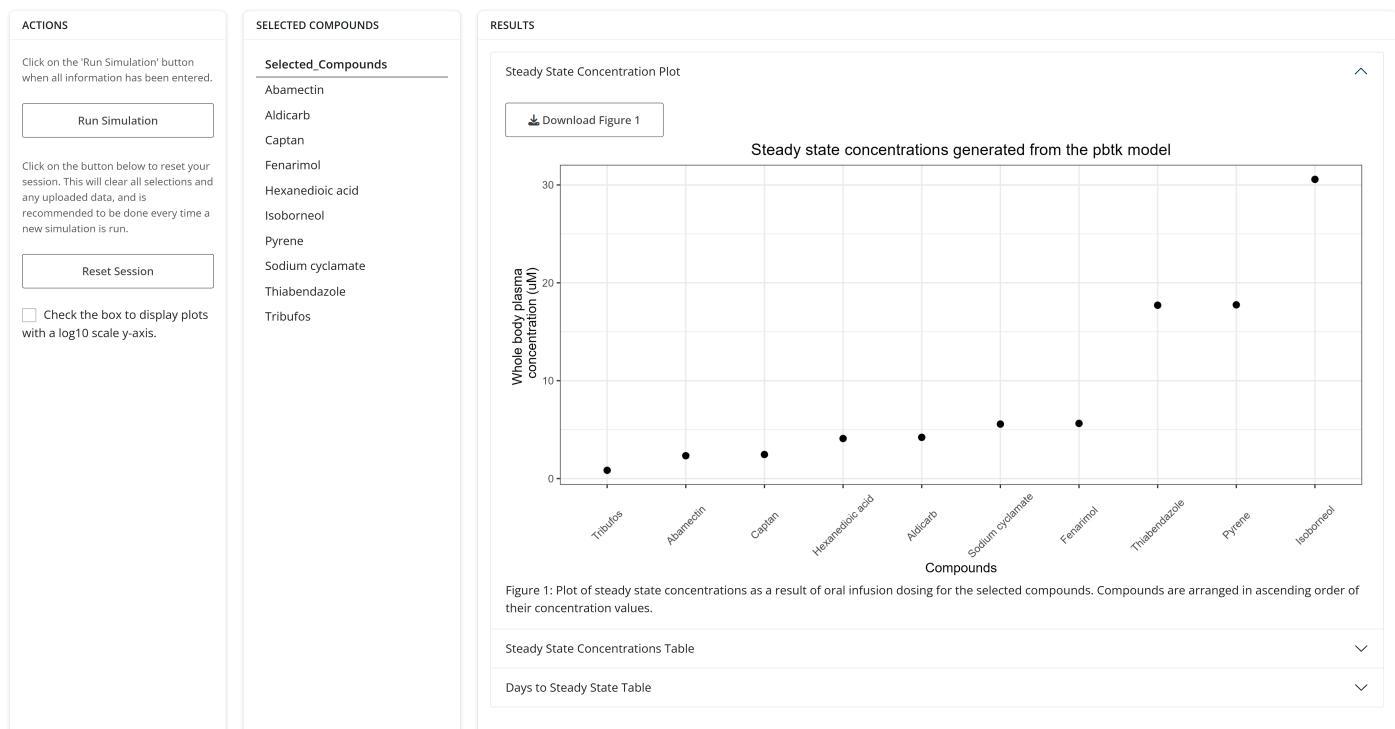
## Run Simulation Tab

Now that we've completed all selections and the compounds we selected appear under the **Selected Compounds** card, we hit the *Run Simulation* button under the **Actions** card as shown in the image below. The simulation will take a few moments to complete.

ACTIONS	SELECTED COMPOUNDS	RESULTS
Click on the 'Run Simulation' button when all information has been entered.  <input type="button" value="Run Simulation"/>	<u>Selected_Compounds</u> Abamectin Aldicarb Captan Fenarimol Hexanedioic acid Isoborneol Pyrene Sodium cyclamate Thiabendazole Tribufos	Steady State Concentration Plot  Steady State Concentrations Table  Days to Steady State Table

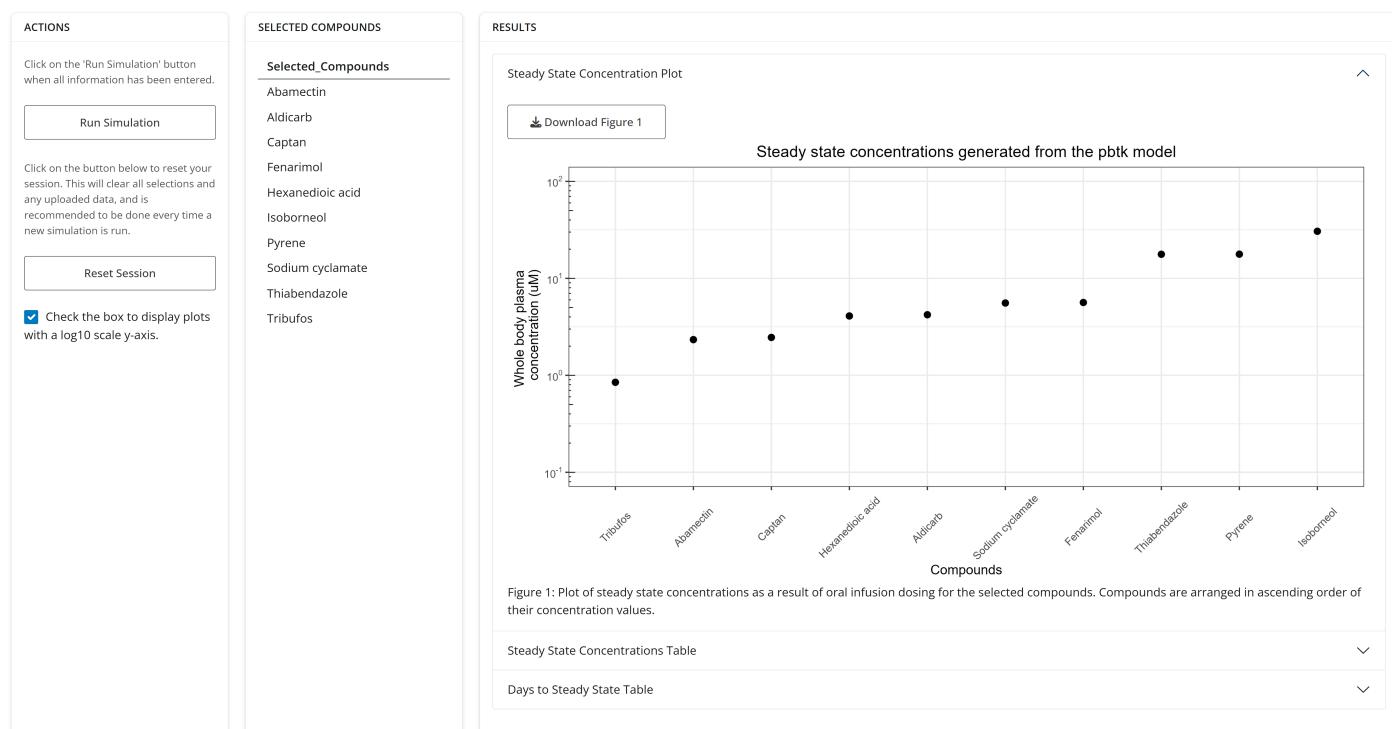
The run simulation tab ready for simulation for example 1.

The image below shows what the *Run Simulation* tab should look like once the simulation is finished. The first drop down bar under the *Results* card shows a single plot of the analytical steady state concentration for each selected compound in ascending order on a linear y-axis. Users have the option to download the steady state concentration plot by clicking the *Download Figure 1* button at the top of the plot.



The run simulation tab with the steady state concentration plot results for example 1.

If the user wants to see the steady state concentrations plot with a log10 y-axis, then the user can check the box at the bottom of the *Actions* tab as shown in blue in the image below. Then, the plot under the first drop down will transform its y-axis to a log10 scale. The user should select this plotting view if there are a vast difference of magnitudes in steady state concentrations across all of the compounds and it is challenging to visualize the smaller concentrations.



The run simulation tab with the steady state concentration plot results with log10 y-scale for example 1.

The second drop down tab contains a table with the numerical values of the steady state concentrations that were plotted in the previous tab. This table is available for download if the user clicks the *Download Table 1* button at the top of the tab. The user is also able to download the simulation parameters used to generate the steady state concentrations by clicking the *Download Simulation Parameters* button.

**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

- Abamectin
- Aldicarb
- Captan
- Fenarimol
- Hexanedioic acid
- Isoborneol
- Pyrene
- Sodium cyclamate
- Thiabendazole
- Tribufos

**RESULTS**

Steady State Concentration Plot

Steady State Concentrations Table

[Download Table 1](#) [Download Simulation Parameters](#)

Show 10 entries

CompoundName	SteadyState
1 Tribufos	0.8472
2 Abamectin	2.333
3 Captan	2.459
4 Hexanedioic acid	4.093
5 Aldicarb	4.213
6 Sodium cyclamate	5.566
7 Fenarimol	5.636
8 Thiabendazole	17.71
9 Pyrene	17.75
10 Isoborneol	30.57

Showing 1 to 10 of 10 entries

Table 1: Table of the steady state concentrations (uM) as a result of oral infusion dosing for the selected compounds in the selected compartment. Compounds are arranged in ascending order of their concentration values.

**Days to Steady State Table**

The run simulation tab with the steady state concentrations table results for example 1.

The final drop down tab under the *Results* card contains a table of steady state characteristics for each of the compounds. The simulation that generates this table determines the number of days (CssDay) it takes for the compound to come within a certain percentage of analytical steady state value from the table in the tab above, the average concentration on the last day of the simulation (AvgConc), the ratio of the average concentration to the analytical steady state concentration (RatioAvgAnalytical), and the maximal concentration of the simulation (MaxConc). The user can download this table by clicking the *Download Table 2* button at the top of the tab. The table should look like that in the image below.

**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
Abamectin
Aldicarb
Captan
Fenarimol
Hexanedioic acid
Isoborneol
Pyrene
Sodium cyclamate
Thiabendazole
Tribufos

**RESULTS**

Steady State Concentration Plot

Steady State Concentrations Table

Days to Steady State Table

**Download Table 2**

Show 10 entries

Search:

CompoundName	CssDay	AvgConc	RatioAvgAnalytical	MaxConc
1 Hexanedioic acid	6	4	0.9715	5.052
2 Aldicarb	8	4.16	0.9863	4.729
3 Tribufos	8	0.8371	0.9864	1.035
4 Sodium cyclamate	10	5.452	0.9779	6.72
5 Captan	16	2.44	0.9912	2.713
6 Fenarimol	20	5.581	0.9903	5.869
7 Thiabendazole	20	17.36	0.9802	18.03
8 Abamectin	80	2.331	0.9988	2.349
9 Isoborneol	119	30.54	0.9988	30.99
10 Pyrene	125	17.41	0.9808	17.59

Showing 1 to 10 of 10 entries

Table 2: Table of steady state (SS) characteristics. CssDay represents the number of days it takes for the model to reach the analytical plasma SS concentration or the fractional change of daily SS plasma concentration is below the set threshold, AvgConc represents the average plasma concentration ( $\mu\text{M}$ ) on the final day of the simulation, RatioAvgAnalytical represents the fraction of the analytical SS plasma concentration reached on CssDay, and MaxConc is the maximum plasma concentration ( $\mu\text{M}$ ) of the simulation.

The run simulation tab with the days to steady state table results for example 1.

If the user wanted to run a new simulation, we suggest clicking the *Reset Session* button under the *Actions* card, which will reset all parameter values and return the user to the starting interface.

## Example 2

In this example, let's say we want to generate human steady state blood concentrations in the liver for a constant daily dose of 2 mg/kg BW using the 1 compartment model without in silico generated parameters for hepatic clearance, fraction unbound in plasma, and caco-2 permeability. Suppose we want to generate these concentrations for the following compounds: Valproic Acid (CAS: 99-66-1), Endosulfan (CAS: 115-29-7), Abamectin (CAS: 71751-41-2), and two fictional chemicals (so we can practice uploading chemicals via a CSV file to the dashboard).

## General Parameters Tab

We select the same parameters for each of the drop downs on the *General Parameters* tab as in Example 1, shown below. Now, the user can continue to the *Model Specifications* tab.

<b>INSTRUCTIONS</b> <p>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.</p> <p>ToCS provides four outputs: 1) Concentration-time profiles (returns chemical concentrations in body compartments over time), 2) Steady state (SS) concentration (returns SS concentrations in body compartments from an oral infusion), 3) In vitro to in vivo extrapolation (IVIVE) (returns oral equivalent doses to in vitro bioactive concentrations), 4) Parameter calculations (returns elimination rates, volumes of distribution, tissue to unbound plasma partition coefficients, half-lives, and total plasma clearances).</p> <p>This application uses the U.S. EPA's R package 'httk'. For more information on ToCS and 'httk', please refer to the following links.</p> <p><a href="#">Vignettes (ToCS tutorials)</a></p> <p><a href="#">Report ToCS issues/suggestions</a></p> <p><a href="#">httk publication</a></p> <p><a href="#">httk CRAN webpage</a></p>	<b>OUTPUT</b> <p>Select the desired output.</p> <p>Steady state concentrations</p>	<b>SPECIES</b> <p>Select the species to analyze.</p> <p>Human</p>
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The completed general parameters tab for example 2.

## Model Specifications Tab

Under the *Dosing* card, we enter 2 into the text box for the 2 mg/kg BW dose. Under the *Model* card, we select *1compartment* for the simulation model with the first drop down menu. For the second drop down, we select *No* since we do not want to include compounds that use in silico parameters in place of missing in vitro parameters. The completed *Model Specifications* card should look like the image below.

**MODEL**

Select a model to simulate.

1compartment

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

**DOSING**

Enter the total daily dose (in mg/kg BW).

2

The completed model specifications tab for example 2.

## Compound Selection Tab

We keep the first drop menu in the *Preloaded Compounds* card on *Choose from all available chemicals* and then in the second drop down menu, we search for all five compounds but only three (valproic acid, endosulfan, and abamectin) are available. The remaining two, Chemical1 and Chemical2, will have to be uploaded under the *Uploaded Compounds* card. Therefore, we upload the chemical information for the two chemicals by copying the SampleCSV file in the *Uploaded Compound File Folder* under the *Instructions* card and entering the appropriate chemical information for each compound. See the *Introduction to ToCS* vignette for more information on upload instructions. For the purpose of this example, we use fictional chemical data and upload the following csv file by clicking *Browse* under the *Uploaded Compounds* card.

A csv file with information for two fictional chemicals.

Compound	CAS	CAS.Checksum	DTXSID	Formula	All.Compound.Names	logHenry
Chemical1	111-11-1	NA	DTXSID11111111	NA	NA	NA
Chemical2	222-22-2	NA	DTXSID22222222	NA	NA	NA

Once we have the three compounds selected under the *Preloaded Compounds* card and the two compounds uploaded under the *Uploaded Compounds* card (with the csv file name CSV\_SSvignette.csv), we then proceed to the next tab. The final *Compound Selection* tab should look like the image below.

The screenshot shows the 'Compound Selection' tab of the ToCS interface. The top navigation bar includes links for General Parameters, Model Specifications, Compound Selection (which is active and underlined), Advanced (Optional) Parameters, and Run Simulation. The main content area is divided into three sections: 'INSTRUCTIONS', 'PRELOADED COMPOUNDS', and 'UPLOADED DATA'. The 'INSTRUCTIONS' section contains a link to 'Uploaded Physical-Chemical Data File Folder'. The 'PRELOADED COMPOUNDS' section has a dropdown menu set to 'Choose from all available chemicals'. Below it is a search input field containing the text '99-66-1, Valproic acid | 115-29-7, Endosulfan | 71751-41-2, Abamectin'. The 'UPLOADED DATA' section shows a file named 'CSV\_vignettes.csv' with a 'Browse...' button and an 'Upload complete' message.

The completed compound selection tab for example 2.

## Advanced (Optional) Parameters Tab

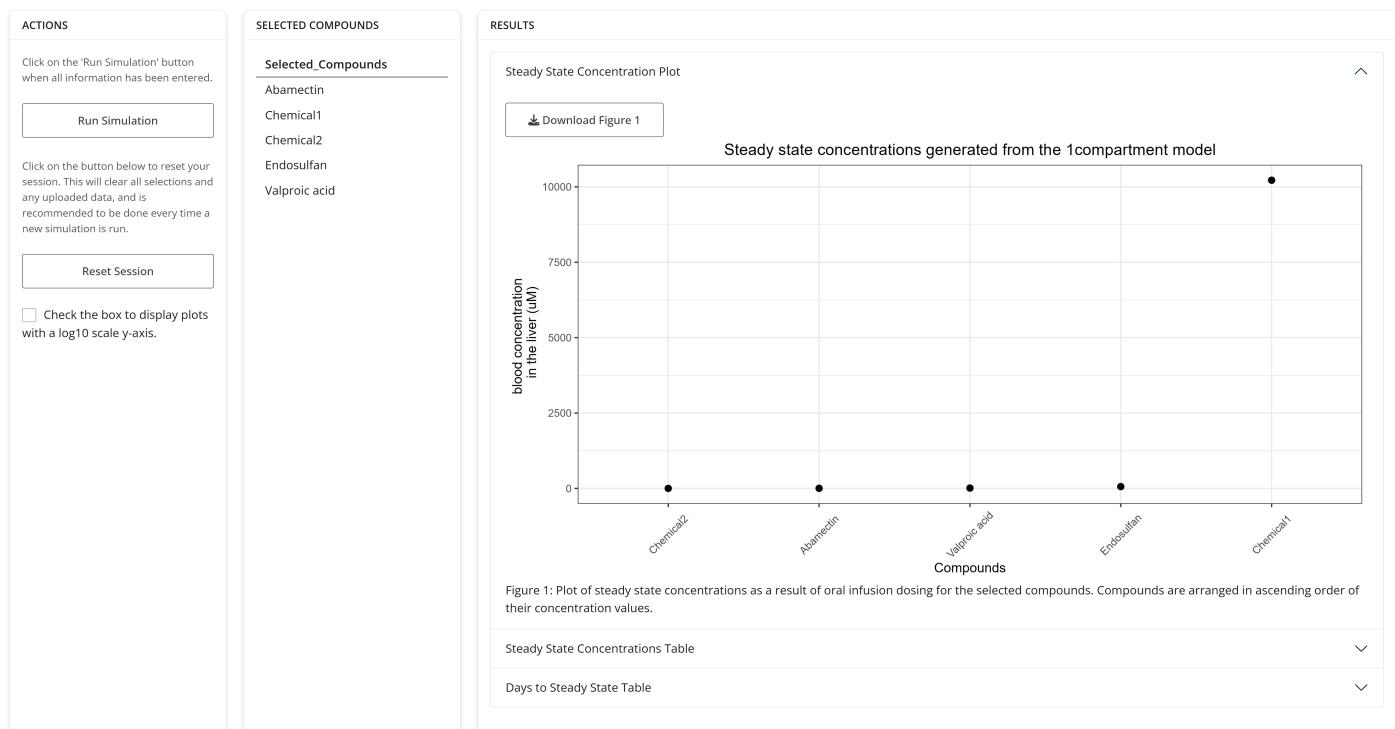
Since we want the steady state blood concentrations in the liver, we customize the second and third drop down menus under the *Output Specification* cards to be *blood* and *liver*, respectively. There are no other customizations we want to make on this page, so the final *Advanced Parameters* page should look like the image below.

<b>MODEL CONDITIONS</b>	<b>MODEL SOLVER</b>	<b>BIOAVAILABILITY</b>	<b>OUTPUT SPECIFICATION</b>
Select whether protein binding is taken into account in liver clearance.  Yes, include protein binding (default)	No options for this category.	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 the output concentration units.  uM  Select the output concentration type.  blood  Select a tissue you want the output concentration in. Leave on 'NULL' if the whole body concentration is desired.  liver
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)	
Enter the p-value threshold for the in vitro intrinsic hepatic clearance rate where clearance assay results with p-values above this threshold are set to zero.  0.05		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)	
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 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)	

The completed advanced parameters tab for example 2.

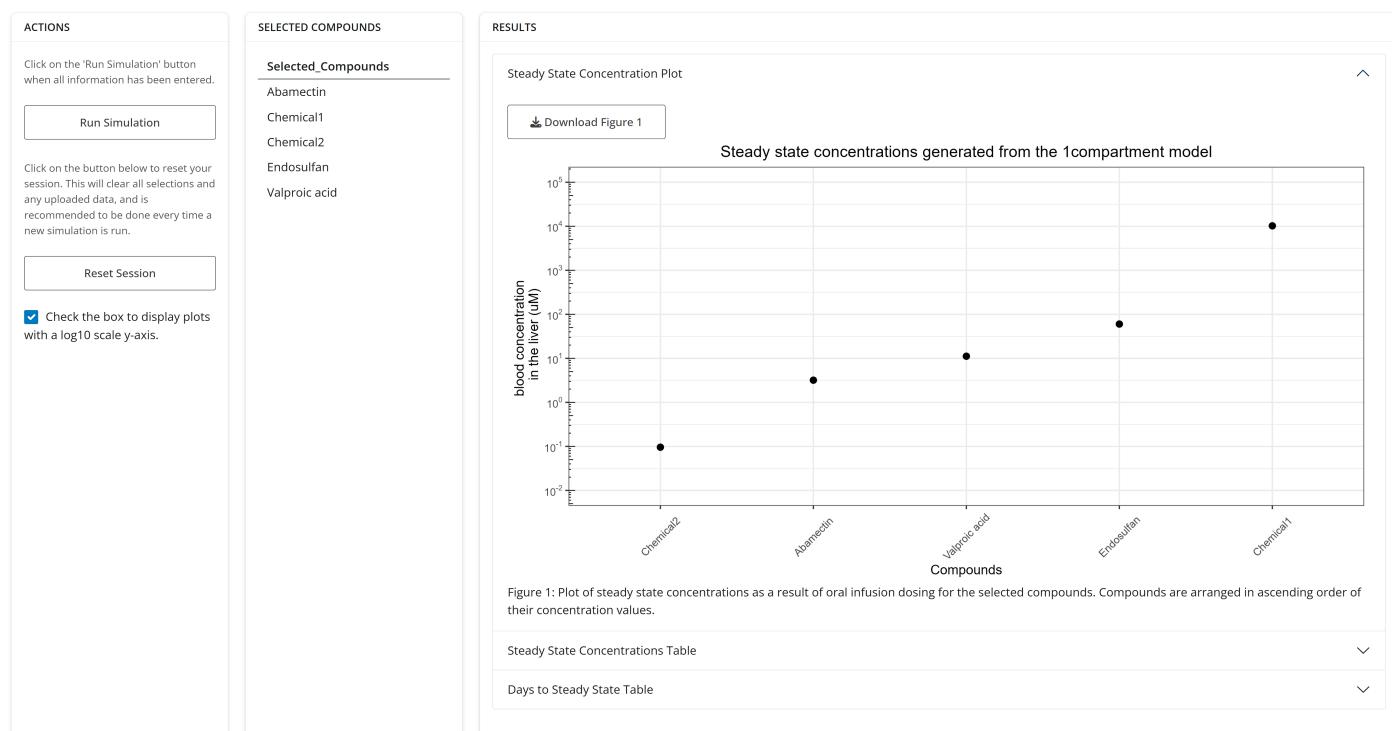
## Run Simulation Tab

Now that all user selections have been made and all selected compounds appear under the *Selected Compounds* card, we are ready to run the simulation. So, we hit the *Run Simulation* button and when the simulation is complete, the results should look like the image below. The plot shows the steady state concentration for all five selected compounds with the user option to download the figure by clicking the *Download Figure 1* button.



The steady state concentration plot with a linear axis for example 2.

Now, given that the outputted plot doesn't provide much visual information since four of the five compound steady state concentrations are significantly smaller than the largest one, we alter this figure by checking the bottom box under the *Actions* card, which will change the y-axis to a log10 scale as shown in the image below. This new plot provides a much clearer visual as to the steady state concentration value for each of the compounds. Note that the log10 scale checkbox can be checked before the user hits the *Run Simulation* button as well.



The steady state concentration plot with a log10 y-axis for example 2.

The second drop down tab under the *Results* card shows a table of the steady state concentrations plotted in the first tab, as seen in the image below. The user has the option to download this table by clicking the *Download Table 1* button directly under the tab. The concentrations are shown in ascending order.

**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

- Abamectin
- Chemical1
- Chemical2
- Endosulfan
- Valproic acid

**RESULTS**

Steady State Concentration Plot

Steady State Concentrations Table

[Download Table 1](#) [Download Simulation Parameters](#)

Show 10 entries

CompoundName	SteadyState
1 Chemical2	0.09579
2 Abamectin	3.188
3 Valproic acid	11.16
4 Endosulfan	60.04
5 Chemical1	10220

Showing 1 to 5 of 5 entries

Table 1: Table of the steady state concentrations (uM) as a result of oral infusion dosing for the selected compounds in the selected compartment. Compounds are arranged in ascending order of their concentration values.

Days to Steady State Table

The steady state concentrations table for example 2.

The final tab under the *Results* card shows steady state characteristics for each compound, as seen in the image below. The description for each of the columns in this table is explained in Example 1 and in the figure caption. The table can be downloaded by the user if the user clicks the *Download Table 2* button.

**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

- Abamectin
- Chemical1
- Chemical2
- Endosulfan
- Valproic acid

**RESULTS**

Steady State Concentration Plot

Steady State Concentrations Table

Days to Steady State Table

**Download Table 2**

Show 10 entries

Search: \_\_\_\_\_

CompoundName	CssDay	AvgConc	RatioAvgAnalytical	MaxConc
1 Chemical2	5	0.09625	0.9381	0.2028
2 Valproic acid	7	14.66	0.9688	20.26
3 Abamectin	97	4.66	0.9986	4.724
4 Endosulfan	573	71.76	0.9997	71.9
5 Chemical1	5896	13010	0.8679	13010

Showing 1 to 5 of 5 entries

Table 2: Table of steady state (SS) characteristics. CssDay represents the number of days it takes for the model to reach the analytical plasma SS concentration or the fractional change of daily SS plasma concentration is below the set threshold, AvgConc represents the average plasma concentration (uM) on the final day of the simulation, RatioAvgAnalytical represents the fraction of the analytical SS plasma concentration reached on CssDay, and MaxConc is the maximum plasma concentration (uM) of the simulation.

Previous 1 Next

The days to steady state table for example 2.

As with the previous example, we suggest that the user clicks the *Reset Session* button if they want to run another simulation.