

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.

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.

Select

Must not be equal to Select.

SPECIES

Select the species to analyze.

Select

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.

Toxicokinetic Chemical Simulator (ToCS)

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INSTRUCTIONS

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OUTPUT

Select the desired output.

Steady state concentrations

SPECIES

Select the species to analyze.

Human

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 *pbtik* for the first drop down to use the pbtik 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.

MODEL

Select a model to simulate.

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.

Yes, load in silico parameters

DOSING

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

1

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).

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

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, Thiabendazole
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.

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

MODEL CONDITIONS

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.

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

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

0.0001

MODEL SOLVER

No options for this category.

BIOAVAILABILITY

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

1.6

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

Use the Caco2.Pab value selected above (default) ▼

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

Use the Caco2.Pab value selected above (default) ▼

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

Do not overwrite in vivo values (default) ▼

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

OUTPUT SPECIFICATION

Select the output concentration units.

uM ▼

Select the output concentration type.

plasma ▼

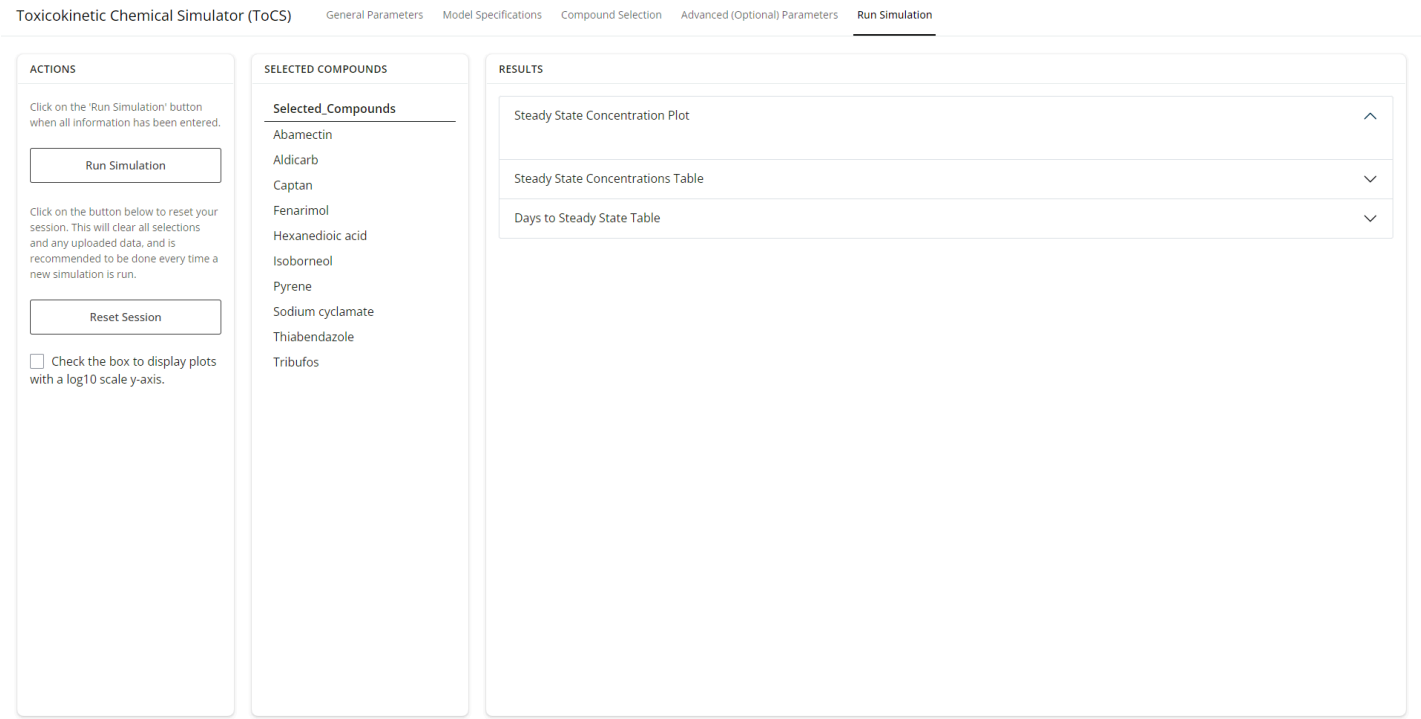
Select a tissue you want the output concentration in. Leave on 'NULL' if the whole body concentration is desired.

NULL ▼

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.



The completed compound selection tab for example 1 showing all selected compounds.

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.

RESULTS

Steady State Concentration Plot

Download Figure 1

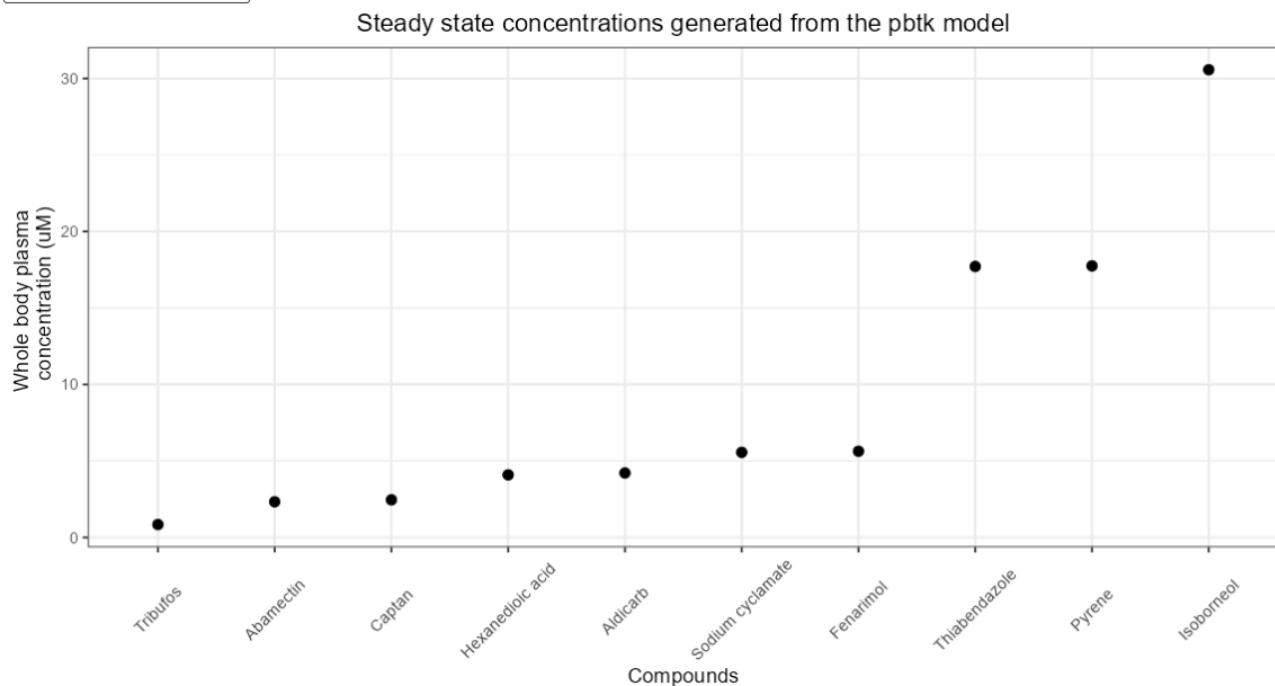


Figure 1: Plot of the long-term constant infusion (steady state) concentrations for the selected compounds. Compounds are arranged in ascending order of their concentration values.

Steady State Concentrations Table

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.

RESULTS

Steady State Concentration Plot

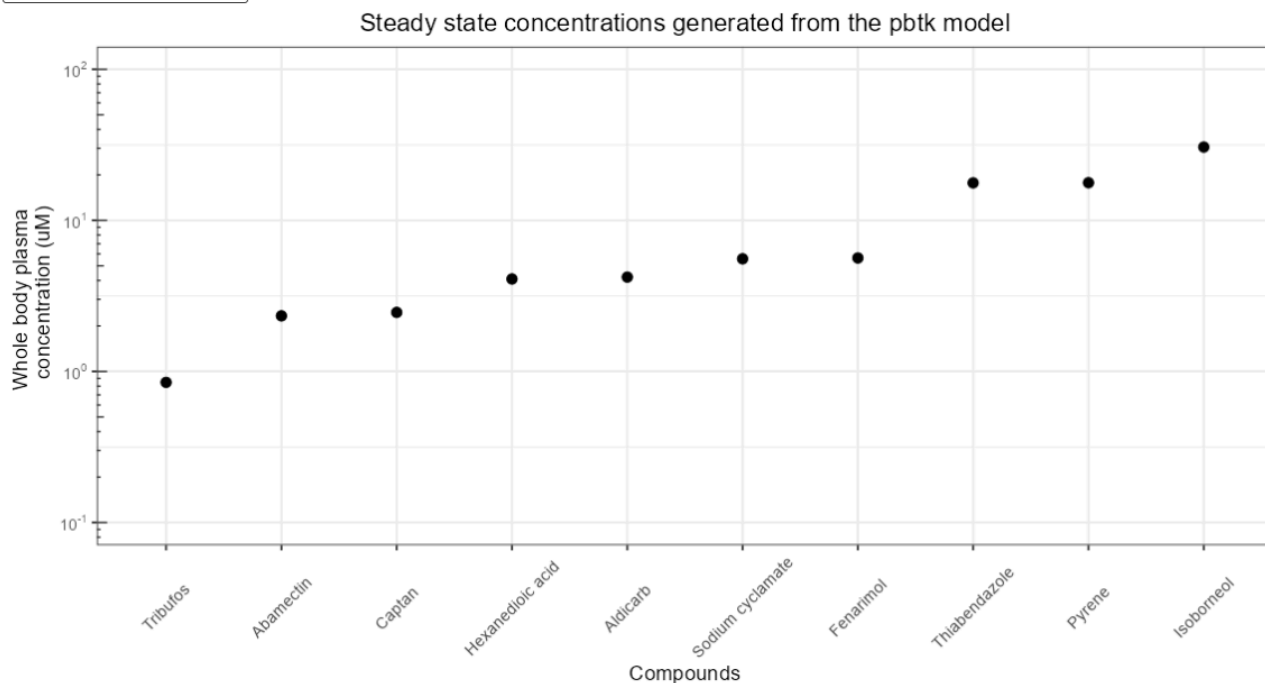
[Download Figure 1](#)

Figure 1: Plot of the long-term constant infusion (steady state) concentrations for the selected compounds. Compounds are arranged in ascending order of their concentration values.

[Steady State Concentrations Table](#)

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.

RESULTS

Steady State Concentration Plot

Steady State Concentrations Table

Download Table 1

Download Simulation Parameters

Show 10 entries

Search:

	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	5.638
8	Thiabendazole	17.71
9	Pyrene	17.75
10	Isoborneol	30.57

Showing 1 to 10 of 10 entries

Previous 1 Next

Table 1: Table of the long-term constant infusion (steady state) concentrations (uM) for the selected compounds. Compounds are arranged in ascending order of their concentration values.

Back 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.

RESULTS

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	5.583	0.9903	5.848
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

Previous 1 Next

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.

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 1compartment 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), Titanium Dioxide (CAS: 13463-67-7), and Advantame (CAS: 714229-20-6).

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.

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.

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OUTPUT

Select the desired output.

Steady state concentrations ▾

SPECIES

Select the species to analyze.

Human ▾

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, titanium dioxide and advantame, will have to be uploaded under the *Uploaded Compounds* card. Therefore, we upload the chemical information for titanium dioxide and advantame 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 fake chemical data and upload the following csv file by clicking *Browse* under the *Uploaded Compounds* card.

A csv file with chemical information for titanium dioxide and advantame. Data other than the chemical identifiers are

Compound	CAS	CAS.Checksum	DTXSID	Formula	All.Compound.Names	logHenry
Titanium Dioxide	13463-67-7	NA	DTXSID3021352	NA	NA	NA
Advantame	714229-20-6	NA	DTXSID00991787	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 Toxicokinetic Chemical Simulator (ToCS). The interface is divided into three main sections: INSTRUCTIONS, PRELOADED COMPOUNDS, and UPLOADED DATA.

INSTRUCTIONS: Contains a link to 'Uploaded Physical-Chemical Data File Folder'.

PRELOADED COMPOUNDS: Includes a dropdown menu 'Choose from all available chemicals' and a list of selected compounds: 99-66-1, Valproic acid; 115-29-7, Endosulfan; and 71751-41-2, Abamectin.

UPLOADED DATA: Includes a 'Browse...' button and a file named 'CSV_SSvignette.csv' with an 'Upload complete' status.

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.

MODEL CONDITIONS	MODEL SOLVER	BIOAVAILABILITY	OUTPUT SPECIFICATION
<p>Select whether protein binding is taken into account in liver clearance.</p> <p>Yes, include protein binding (default) ▾</p> <p>Select whether to adjust the chemical fraction unbound in presence of plasma proteins for lipid binding.</p> <p>Yes, adjust the fraction of unbound plasma (default) ▾</p> <p>Select whether to use regressions when calculating partition coefficients.</p> <p>Use regressions (default) ▾</p> <p>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.</p> <p>0.05</p> <p>Enter the minimum acceptable chemical fraction unbound in presence of plasma proteins. All values below this will be set to this value.</p> <p>0.0001</p>	<p>No options for this category.</p>	<p>Enter a default value for the Caco-2 apical-to-basal membrane permeability (denoted Caco2.Pab, 10^{-6} cm/s).</p> <p>1.6</p> <p>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.</p> <p>Use the Caco2.Pab value selected above (default) ▾</p> <p>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.</p> <p>Use the Caco2.Pab value selected above (default) ▾</p> <p>Select whether to overwrite in vivo F_abs and F_gut data (if available).</p> <p>Do not overwrite in vivo values (default) ▾</p> <p>Select whether to keep F_abs and F_gut at 100% availability (which overwrites all other bioavailability parameter settings).</p>	<p>Select the output concentration units.</p> <p>uM ▾</p> <p>Select the output concentration type.</p> <p>blood ▾</p> <p>Select a tissue you want the output concentration in. Leave on 'NULL' if the whole body concentration is desired.</p> <p>liver ▾</p>

The completed advanced parameters tab for example 2.

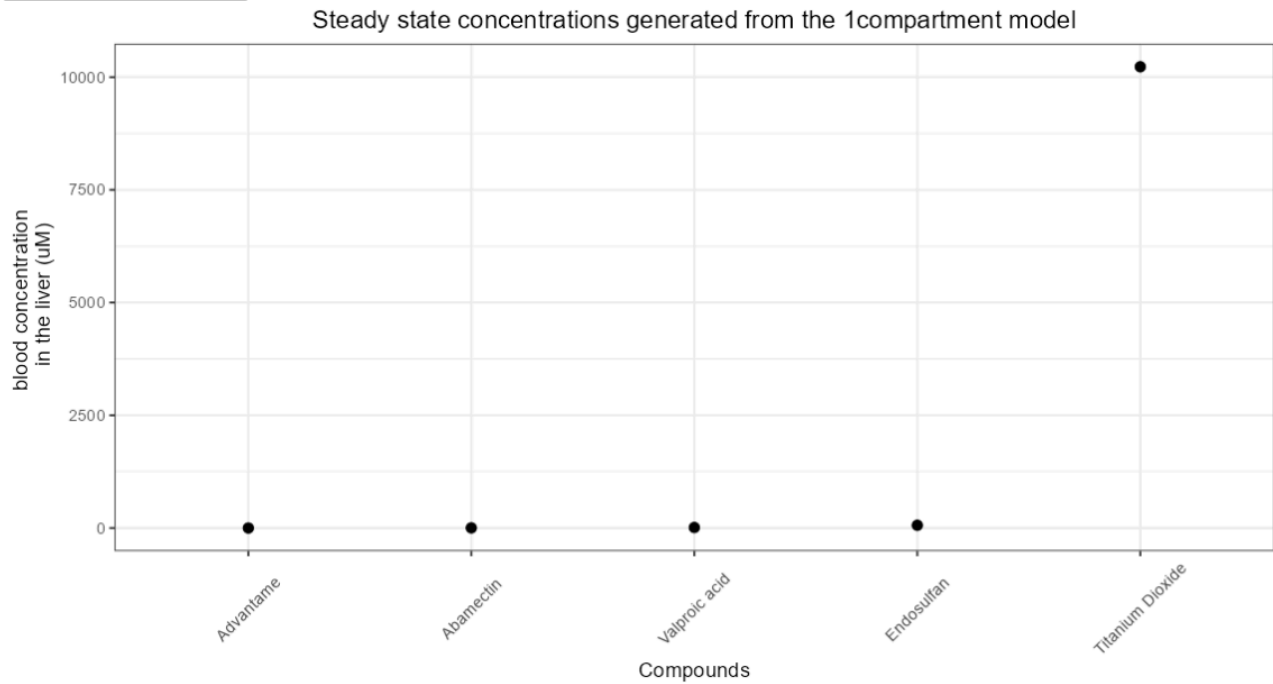
Run Simulation Tab

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.

RESULTS

Steady State Concentration Plot

Download Figure 1



Steady State Concentrations Table

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.

RESULTS

Steady State Concentration Plot

[Download Figure 1](#)

Steady state concentrations generated from the 1compartment model

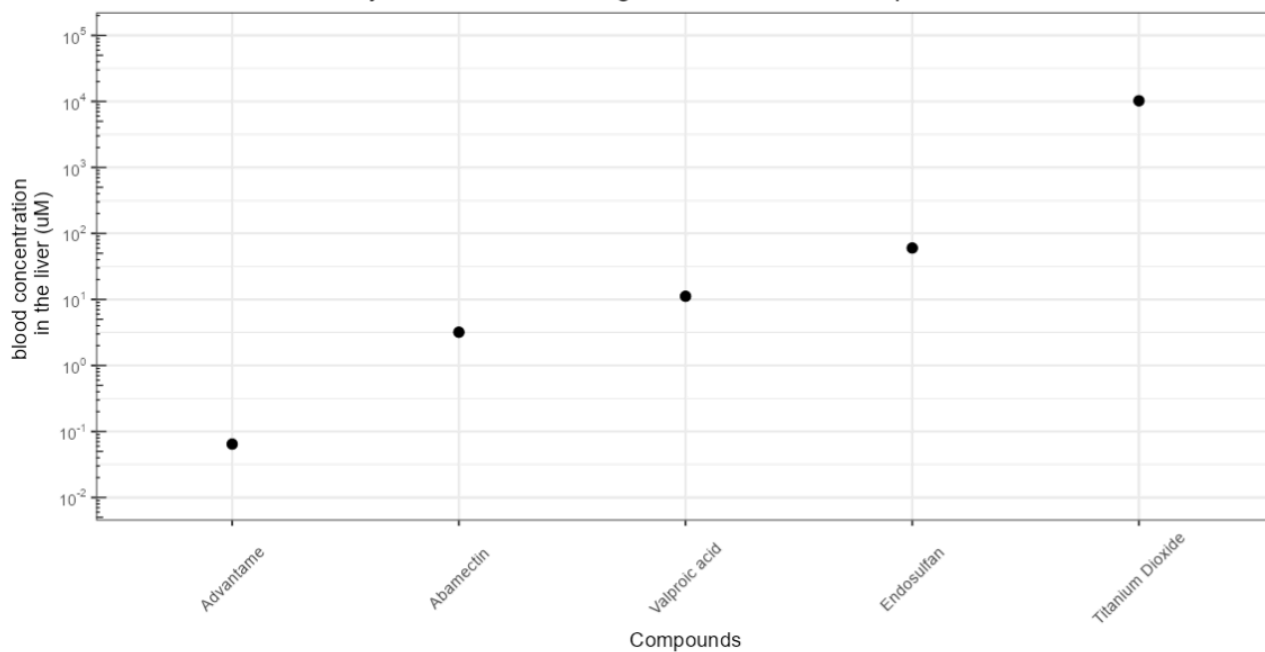


Figure 1: Plot of the long-term constant infusion (steady state) concentrations for the selected compounds. Compounds are arranged in ascending order of their concentration values.

Steady State Concentrations Table

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.

RESULTS

Steady State Concentration Plot



Steady State Concentrations Table



Download Table 1

Download Simulation Parameters

Show 10 entries

Search:

	CompoundName	SteadyState
1	Advantame	0.06434
2	Abamectin	3.188
3	Valproic acid	11.16
4	Endosulfan	60.04
5	Titanium Dioxide	10230

Showing 1 to 5 of 5 entries

Previous 1 Next

Table 1: Table of the long-term constant infusion (steady state) concentrations (uM) for the selected compounds. 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.

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	Advantame	1	0.06465	0.9381	0.1362
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	Titanium Dioxide	3270	10130	0.6747	10130

Showing 1 to 5 of 5 entries

Previous 1 Next

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.

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.