

Concentration-Time Profile 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 three examples that use the ToCS app to generate concentration-time profiles, 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 and underlined), 'Model Specifications', 'Compound Selection', 'Advanced (Optional) Parameters', and 'Run Simulation'. Below the navigation bar, there are three main input sections:

- INSTRUCTIONS:** A text area containing 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." It also describes the four outputs: Concentration-time profiles, Steady state (SS) concentration, In vitro to in vivo extrapolation (IVIVE), and Parameter calculations.
- OUTPUT:** A dropdown menu labeled "Select the desired output." with the option "Select". A note below says "Must not be equal to Select."
- SPECIES:** A dropdown menu labeled "Select the species to analyze." with the option "Select". A note below says "Must not be equal to Select."

At the bottom of the interface, there are links for further information: "Vignettes (ToCS tutorials)", "Report ToCS issues/suggestions", "httk publication", and "httk CRAN webpage".

The opening interface to the ToCS app.

Model States

Before jumping into several examples, we define the output states that users will see in plots and tables generated by ToCS for this module. Below, we list the model chosen and all of its output states.

- 1compartment
 - Agutlumen = Amount of chemical in the gut lumen
 - Ccompartment = Plasma concentration of chemical in main absorption compartment
 - Ametabolized = Amount of chemical metabolized by the main compartment
 - AUC = Area under the curve of the Ccompartment compartment
- 3compartment
 - Aintestine = Amount of chemical in the intestine
 - Cliver = Concentration of chemical in the liver
 - Csyscomp = Concentration of chemical in the systemic compartment
 - Cplasma = Concentration of chemical in the plasma
 - Atubules = Amount of chemical excreted by the systemic compartment through the tubules
 - Ametabolized = Amount of chemical metabolized by the liver
 - AUC = Area under the curve of the Csyscomp compartment
- pbtk
 - Agutlumen = Amount of chemical in the gut lumen
 - Cgut = Concentration of chemical in the gut
 - Cliver = Concentration of chemical in the liver
 - Cven = Concentration of chemical in veins
 - Clung = Concentration of chemical in the lung
 - Cart = Concentration of chemical in arteries
 - Crest = Concentration of chemical in rest of body
 - Ckidney = Concentration of chemical in kidney
 - Cplasma = Concentration of chemical in plasma
 - Atubules = Amount of chemical excreted by the kidney through the tubules
 - Ametabolized = Amount of chemical metabolized by the liver
 - AUC = Area under the curve of the Cplasma compartment
- fetal_pbtk
 - All outputs in the pbtk model for maternal concentrations and amounts plus:
 - Cadipose = Concentration of chemical in adipose tissue
 - Rblood2plasma = Dynamic maternal ratio of blood to plasma (unitless)
 - fAUC = Area under the curve of the Cfplasma compartment
 - Cplacenta = Concentration of chemical in placenta
 - Cf liver = Concentration of chemical in fetal liver
 - Cfven = Concentration of chemical in fetal veins
 - Cfart = Concentration of chemical in fetal arteries
 - Cfgut = Concentration of chemical in fetal gut
 - Cflung = Concentration of chemical in fetal lung
 - Cfrest = Concentration of chemical in fetal rest of body
 - Cfthyroid = Concentration of chemical in fetal thyroid
 - Cfkidney = Concentration of chemical in fetal kidney
 - Cfbrain = Concentration of chemical in fetal brain
 - Cfplasma = Concentration of chemical in fetal plasma
 - Rfblood2plasma = Dynamic fetal ratio of blood to plasma (unitless)
- full_pregnancy

- All outputs in the fetal_pbtk model for plus:
- Cthyroid = Concentration of chemical in maternal thyroid
- Aconceptus = Amount of chemical in conceptus (embryo)
- Cconceptus = Concentration of chemical in conceptus (embryo)

Amount outputs have units of umol, while concentration outputs are in uM. The area under the curve outputs have units uM*days. Any other units are specified above when defined. The user can also check the figure captions for units. If the user wants further details on the models and their formulations, they are encouraged to check out the htk documentation [here] (<https://cran.r-project.org/web/packages/htk/index.html>) (<https://cran.r-project.org/web/packages/htk/index.html>).

Example 1

Let's say we want to run a simulation that outputs human concentration-time profiles over the course of five days for four compounds: Abamectin (CAS: 71751-41-2), Bisphenol-A (CAS: 80-05-7), Cyanazine (CAS: 21725-46-2), and Dimethoate (CAS: 60-51-5). The simulation will be for a single 5 mg/kg oral exposure of each compound and use the PBTK model without including in silico generated parameters in place of in vitro data.

General Parameters Tab

Since the main output we want is concentration-time profiles, we select *Concentration-time profiles* from the drop down menu under the *Output* card. Under the *Species* card, we select *Human* species. Thus, the completed first tab should look like the page below.

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

- INSTRUCTIONS:** A text area containing instructions for using the simulator, mentioning four types of outputs: concentration-time profiles, steady state concentrations, in vivo extrapolation (IVIVE), and parameter calculations.
- OUTPUT:** A dropdown menu set to "Concentration-time profiles".
- SPECIES:** A dropdown menu set to "Human".

At the top of the page, there are navigation links: Toxicokinetic Chemical Simulator (ToCS), General Parameters (which is active), Model Specifications, Compound Selection, Advanced (Optional) Parameters, and Run Simulation.

Below the main sections, there are additional links for support and documentation:

- [Vignettes \(ToCS tutorials\)](#)
- [Report ToCS issues/suggestions](#)
- [httk publication](#)
- [httk CRAN webpage](#)

A completed opening interface to the ToCS app.

Now, we move on to the *Model Specifications* tab.

Model Specifications Tab

On the *Model* card, we select *pbtk* for the pbtk model on the first drop down menu. Since we do not want to use in silico generated parameters for this simulation, we select *No* for the second drop down menu under the *Model* card. Finally, since we only want to run our simulation for five days, we edit the bottom box in the *Model* card to be 5 instead of the default value of 10. On the *Dosing* card, we leave the first two drop down menus as their default values. For the dosing frequency, we select *Single Dose* from the drop down menu. This prompts the appearance of a textbox where we can input the number of mg/kg to be administered. We change its value to 5 since we want a single 5 mg/kg exposure. Now the *Model Specifications* tab is completed and should look like the image below, so we can proceed to the *Compound Selection* tab.

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

MODEL Select a model to simulate. <input type="text" value="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. <input type="text" value="No, do not load in silico parameters"/> Enter the total simulation time (in days). <input type="text" value="5"/>	DOSING Select the administration method of the compound(s). <input type="text" value="oral"/> Select the units of the administered dose(s). <input type="text" value="mg/kg"/> Select the dosing frequency. <input type="text" value="Single Dose"/> Enter the dose amount administered (in the specified units). <input type="text" value="5"/>
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The completed model specifications tab for the pbtk model with a single oral dose of 5 mg/kg.

Compound Selection Tab

Since we want to simulate four compounds (abamectin, bisphenol-a, cyanazine, and dimethoate), we keep the first drop menu in the *Preloaded Compounds* card on *Choose from all available chemicals* selection and try searching the second drop down menu to see if the program is able to simulate those chemicals with the current data in htk. To see the available compounds, click on the empty box in the center column. By either scrolling or typing in the

textbox, we see that the names of all four compounds are also available, so we select those. Since all of the compounds we need are available, we do not need to upload a CSV file under the *Uploaded Data* card and leave it untouched. Then, we proceed to the next tab.

The screenshot shows the 'Compound Selection' tab of the ToCS interface. At the top, there are tabs for General Parameters, Model Specifications, Compound Selection (which is active), Advanced (Optional) Parameters, and Run Simulation. Below the tabs are three main sections: INSTRUCTIONS, PRELOADED COMPOUNDS, and UPLOADED DATA.

- INSTRUCTIONS:** A text area with 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." Below this is a link: [Uploaded Physical-Chemical Data File Folder](#).
- PRELOADED COMPOUNDS:** A section titled "Select the types of compounds you want to simulate." It includes a dropdown menu "Choose from all available chemicals" and a search input field. Below the input field is a list of selected compounds: "71751-41-2, Abamectin 80-05-7, Bisphenol-a 21725-46-2, Cyanazine 60-51-5, Dimethoate".
- UPLOADED DATA:** A section titled "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." It contains a "Browse..." button and a text field showing "No file selected".

The completed compound selection card for example 1.

Advanced (Optional) Parameters Tab

For simplicity of this example, we will leave all selections and inputs on this tab alone and proceed to the next and final tab.

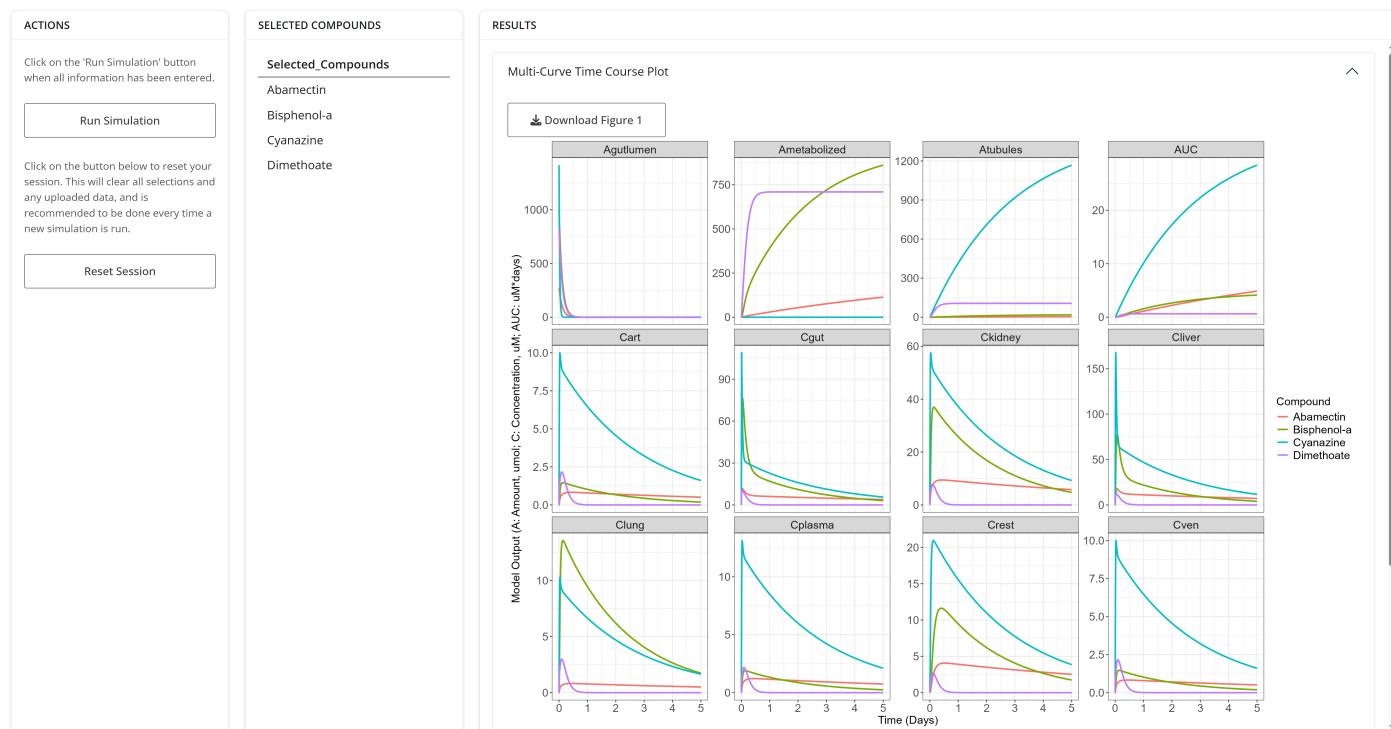
Run Simulation Tab

All input selections are complete and the correct compounds appear under the *Selected Compounds* card, as shown in the image below. Therefore, we hit the *Run Simulation* button under the *Actions* card so ToCS can compute the solution. The output will appear in the *Results* window when complete. Depending on the number of compounds selected to simulate, the results may take several seconds to populate.

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 Bisphenol-a Cyanazine Dimethoate	Multi-Curve Time Course Plot Click here to see definitions of each subplot heading (model compartment) Individual Time Course Plots Time Course Data TK Summary Data

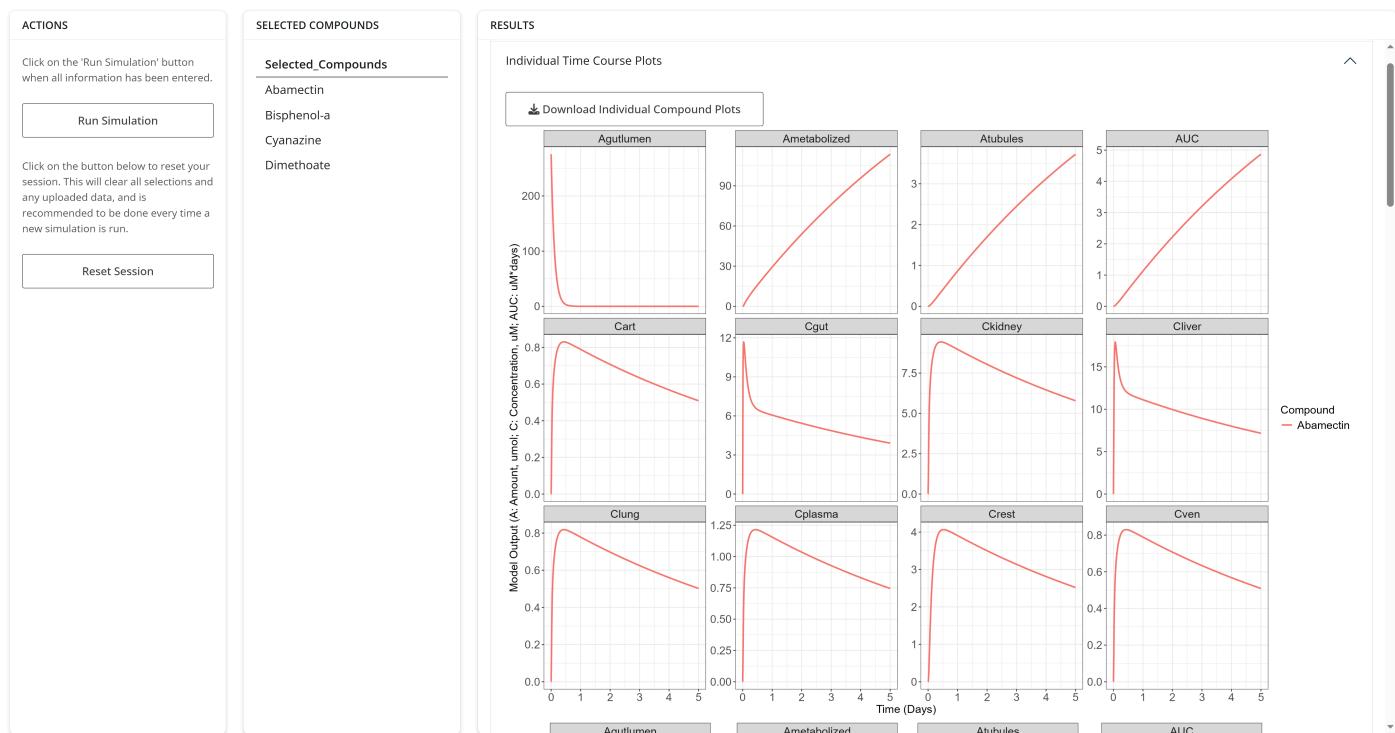
The run simulations tab appearance before the “Run Simulation” button under the *Actions* card is clicked.

The image below shows the first drop down in the *Results* card once the simulation is complete. The user sees the complete time course curves of all four chemicals in each model compartments overlaying each other. The legend for the figure is located in the bottom right corner, and a figure description describing the y-axis of each subplot is located below the figure. The user also has the option to download this figure by clicking *Download Figure 1*.



The multi-curve plot output and download option for example 1.

The second drop down in the *Results* card, as seen below, shows the user the same plots as seen in the first drop down tab but with each compound on a separate plot. The user has the option to download all individual plots as a zip file. A figure caption is also located under the very last plot in this tab.



The individual plots output and download option for example 1.

The third drop down in the *Results* card allows the user to download the time course simulation data that was used to generate the plots in the two drop downs above. The user can also download all of the inputted simulation parameters as well as the chemical data used in the simulations. The interface with these two download buttons is shown below. Opening the bottom drop down in the *Results* card shows a toxicokinetic summary including the Tmax (time to maximal concentration), Cmax (maximal concentration), and AUC (area under the curve) of all simulated compounds within each model compartment. The table is available for download if the user clicks *Download Table 1*.

	Tmax.Abarectin	MaxValue.Abarectin	AUC.Abarectin	Tmax.Bisphenol-a	MaxValue.Bisphenol-a	AUC.Bisphenol-a
Agutlumen	0	275.6	30.96	0	987.4	110.9
Cgut	0.0312	11.68	26.76	0.0416	76.74	62.18
Cliver	0.0624	17.94	48.52	0.0728	78.15	77.19
Cven	0.4264	0.8299	3.327	0.1352	1.467	3.188
Clung	0.4264	0.8184	3.281	0.1352	13.56	29.47
Cart	0.4264	0.8299	3.326	0.1352	1.467	3.188
Crest	0.5096	4.065	16.19	0.3952	11.62	27.89
Ckidney	0.4264	9.432	37.8	0.1352	36.96	80.33
Cplasma	0.416	1.215	4.869	0.1352	1.905	4.139
Atubules	5	3.723	9.998	5	18.1	59.58

The simulation data download feature and the toxicokinetic (TK) summary drop down table for example 1.

If we wanted to run another simulation, we would click the *Reset Session* button under the *Actions* card, 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 run a simulation that outputs rat concentration-time profiles over the course of three days for five compounds: Valproic Acid (CAS: 99-66-1), Benzoic Acid (CAS: 65-85-0), Ethanol (CAS: 64-17-5), and two fictional chemicals: Chemical1 (CAS: 111-11-1), and Chemical2 (CAS: 222-22-2). The simulation will be for three oral exposures a day every eight hours of 2 mg/kg each for each compound. We will use the 3-compartment model without including in silico generated parameters for missing in vitro data.

General Parameters Tab

As with example 1, we select *Concentration-time profiles* as the desired output under the *Output* card. This time, however, we select *Rat* species for the first drop down under the *Species* card. Let's say that, in this example, we only want to use rat in vitro data instead of using human in vitro data for compounds missing rat data. Thus, we select *No* for the second drop down menu under the *Species* card. The first tab should now look like the image below.

INSTRUCTIONS <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>Vignettes (ToCS tutorials)</p> <p>Report ToCS issues/suggestions</p> <p>httk publication</p> <p>httk CRAN webpage</p>	OUTPUT <p>Select the desired output.</p> <p>Concentration-time profiles</p>	SPECIES <p>Select the species to analyze.</p> <p>Rat</p> <p>Do you want to use human in vitro data if in vitro data for the selected species is missing?</p> <p>No</p>
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The opening interface to the ToCS app for example 2.

Model Specifications Tab

On the *Model* card, we select *3compartment* model under the first drop down menu. For the second drop down menu, we select *No* since we do not want to use in silico generated parameters. Finally, we enter *3* in the final box under the *Model* card since we want to run the simulation for three days. On the *Dosing* card, we again leave the first two drop down menus at their default selections. For the dosing frequency (third) drop down menu, we select *Multiple Doses*. A fourth drop down menu will then appear asking whether we want the multiple doses to be evenly distributed throughout the day. Since we want to administer a 2 mg/kg dose every 8 hours, we select *Yes* from the drop down menu. Finally, two additional user options appear. The first one asks the user to specify the amount of chemical exposure per administration, and so we enter *2* into the box. The second one is a slider that has the user specify the frequency of the dose, and so we move the slider to 8 hours. Therefore, the final interface of the *Model Specifications* tab should look like the image below.

MODEL

Select a model to simulate.

Enter the total simulation time (in days).

DOSING

Select the administration method of the compound(s).

Select the units of the administered dose(s).

Select the dosing frequency.

Are equal doses given evenly across a 24 hour period (i.e. 1 mg/kg BW every 8 hours)?

Enter the amount administered during every dose (in the specified units).

Select how often the above dose is administered (every ____ hours).

0 2.5 5 7.5 10 12.5 15 17.5 20 22.5 24

The completed model specifications tab for example 2.

Compound Selection Tab

We first try to search for the compounds we desire to simulate by keeping the first drop menu in the *Preloaded Compounds* card on *Choose from all available chemicals* and then searching through the second drop down menu. Typing in the chemical names or CAS numbers shows that three of the five desired chemicals (valproic acid, benzoic acid, ethanol) are present on the preloaded compounds list and two chemicals are not (Chemical1 and Chemical2). Therefore, we select valproic acid, benzoic acid, and ethanol on the *Preloaded Compounds* card and will have to upload the remaining two compounds under the *Uploaded Data* card. To get the chemical information for Chemical1 and Chemical2 into the program, we copy the *SampleCSV.csv* file in the *Uploaded Physical-Chemical File Folder* under the *Instructions* card and enter 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 Data* card.

A csv file with two fictional chemicals.

Compound	CAS	CAS.Checksum	DTXSID	Formula	All.Compound.Names	logHenry
Chemical1	111-11-1	NA	DTXSID11111111	NA	NA	NA

Compound	CAS	CAS.Checksum	DTXSID	Formula	All.Compound.Names	logHenry
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 Data* card (with the csv file name CSV_vignettes.csv), we then proceed to the next tab. The final *Compound Selection* tab should look like the image below.

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.
99-66-1, Valproic acid | 65-85-0, Benzoic acid | 64-17-5, Ethanol

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... CSV_vignettes.csv Upload complete

The completed compound selection tab for example 2.

Advanced (Optional) Parameters Tab

Suppose that we want to customize parameters other than just the basic simulation information needed. For this example, let's say we want to change the starting amount of each compound in the gut lumen and gut to be 10 umol instead of 0 umol. So, we select *Yes, enter my own initial amounts* under the first drop down menu in the *Model Conditions* card. Consequently, an additional seven text boxes appear under this drop down. Since we only want to change the amounts in the gut lumen and gut, we change the first two text boxes to be 10 instead of 0. There are no other changes we want to make, so we leave the remaining selections as is and proceed to the final tab, *Run Simulation*. The completed advanced parameters page should look like the image below.

MODEL CONDITIONS

Would you like to change the initial compound amount in each compartment from its default value of 0 (no compound in the compartment when the simulation begins)?

Yes, enter my own initial amounts

Enter the initial amount (in umol) of compound(s) in the gut lumen at t = 0.

Enter the initial amount (in umol) of compound(s) in the gut at t = 0.

Enter the initial amount (in umol) of compound(s) in the liver at t = 0.

Enter the initial amount (in umol) of compound(s) in the rest of body at t = 0.

Enter the initial amount (in umol) of compound(s) metabolized at t = 0.

Enter the initial amount (in umol) of compound(s) in the tubules at t = 0.

MODEL SOLVER

Select the ODE solver method. See R documentation on the 'deSolve' function for method details.

lsoda

Enter the number of time steps per hour for the solver to take.

Select the exponent (power of 10) of the relative tolerance for the ODE solver.

Select the exponent (power of 10) of the desired absolute tolerance for the ODE solver.

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)

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)

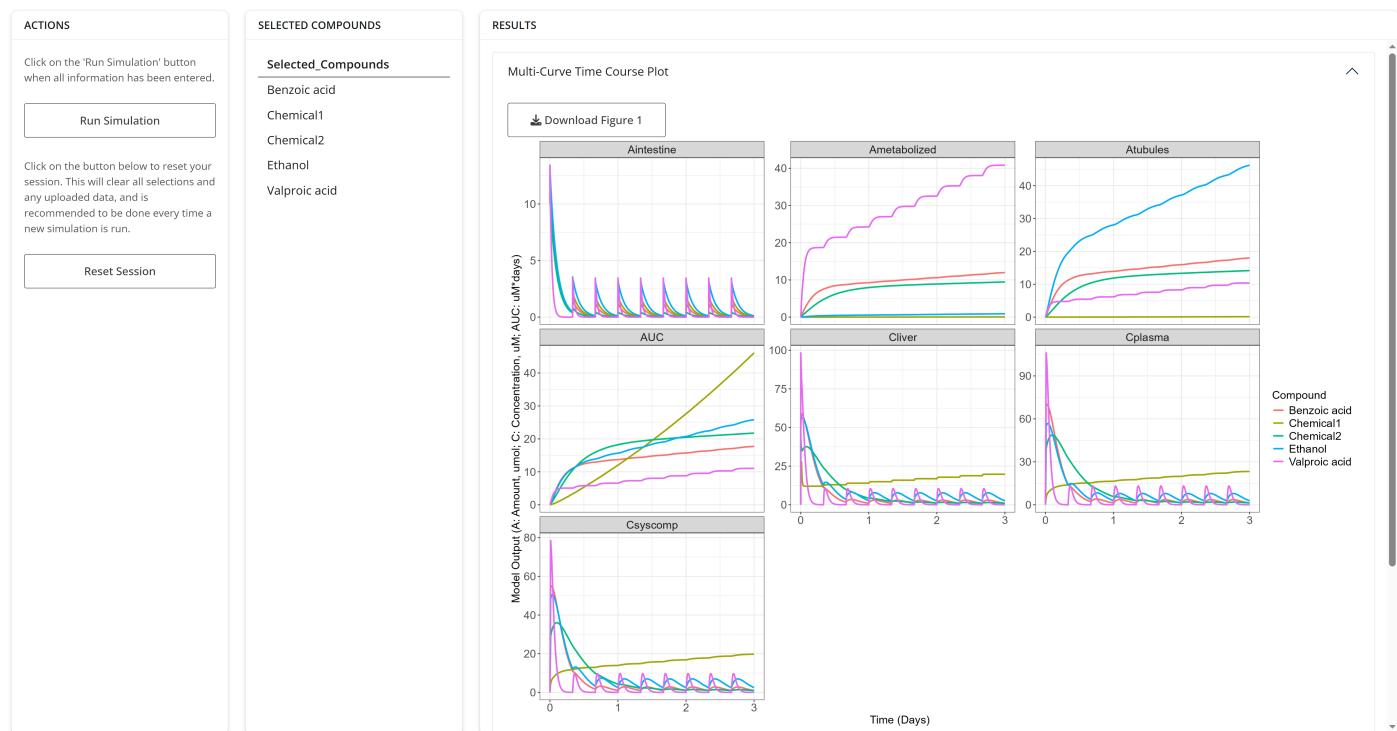
OUTPUT SPECIFICATION

Enter the times (in days) to output concentrations. Leave blank if no specific times are needed. Enter a comma-separated list, such as 0, 1, 2, ... signifying output 0, 1, and 2 days after dosing begins.

The completed advanced parameters tab for example 2.

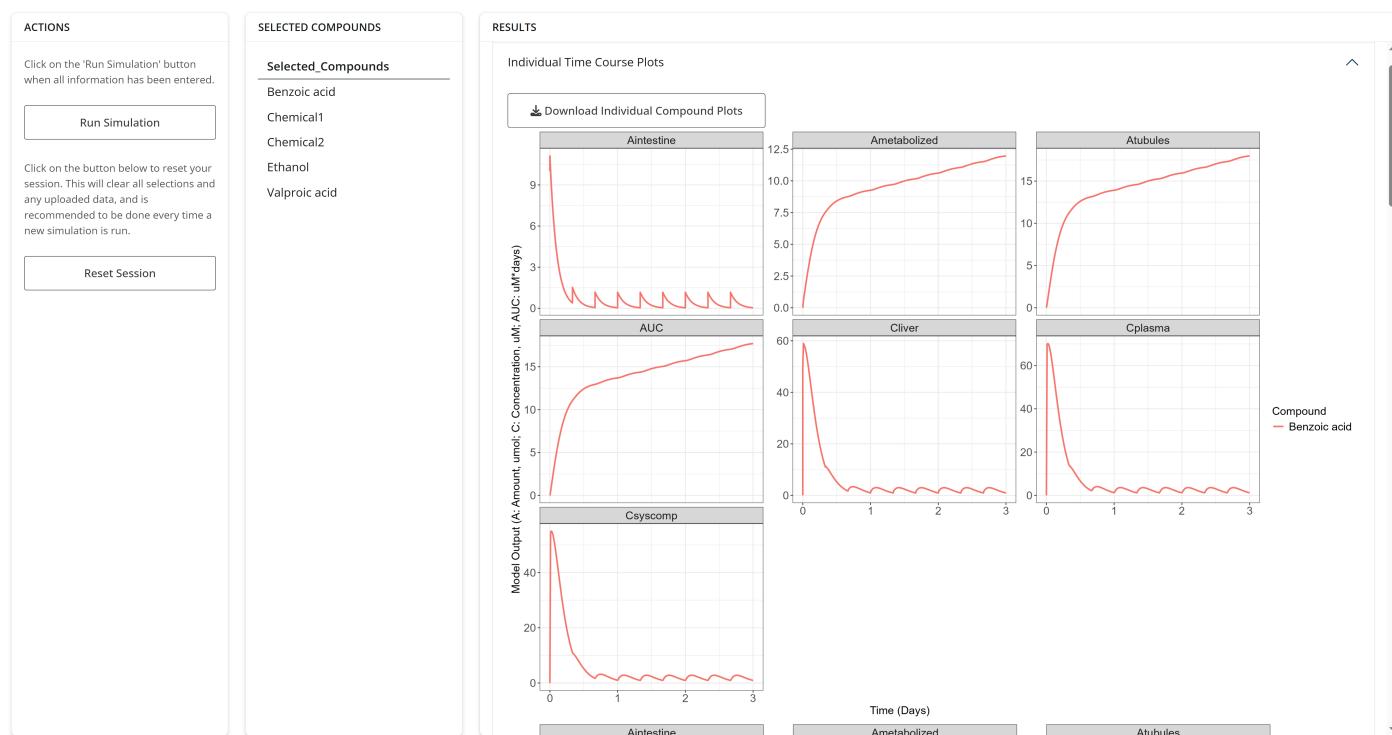
Run Simulation Tab

Now, we've filled out all simulation information and the correct compounds appear under the *Selected Compounds* card. Thus, we click the *Run Simulation* button under the *Actions* card. The image of the completed simulation is shown below. The first tab illustrates the time course plots of all five compounds for each model compartment. The user has the option to download the plot by clicking *Download Figure 1*.



The multi-curve time course plot for example 2.

By closing the *Multi-Curve Time Course Plot* drop down, we view the *Individual Time-Course Plots* drop down and see the same plots as shown in the drop down above but all on a separate plots (one figure per compound). The user can download the individual plots by clicking the *Download Individual Compound Plots* button, where all the plots will download as a zip file.



The individual time course plots for example 2.

The final below shows the user's option to download the simulation results as well as the simulation parameters used to generate the solution. The fourth drop down tab shows a table of simulation summary statistics for each compound in each model compartment. The user has the option to download the table by clicking the *Download Table 1*.

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

SELECTED COMPOUNDS

Selected_Compounds

- Benzoic acid
- Chemical1
- Chemical2
- Ethanol
- Valproic acid

RESULTS

Multi-Curve Time Course Plot

Individual Time Course Plots

Time Course Data

[Download ADME Time Course Data](#) [Download ADME Simulation Parameters](#)

TK Summary Data

[Download Table 1](#)

Show 10 entries

Search:

	Tmax.Benzoic.acid	MaxValue.Benzoic.acid	AUC.Benzoic.acid	Tmax.Chemical1	MaxValue.Chemical1	AUC.Chemical
Aintestine	0.0001	11.12	2.011	0.0001	11.77	2.
Cliver	0.0104	58.96	18.42	0.0104	27.91	4.
Csyscomp	0.0208	55.02	17.54	2.995	19.73	4.
Plasma	0.0208	70.18	22.38	2.985	23.25	5.
Atubules	3	17.99	43	3	0.1411	0.1
Ametabolized	3	11.97	28.68	3	0.05697	0.07
AUC	3	17.72	42.35	3	46.11	6.

Showing 1 to 7 of 7 entries

Table 1: Table of summary statistics (Tmax - time to maximal concentration, MaxValue - maximal amount (A, umol) or concentration (C, uM), AUC - area under the curve ($\mu\text{M}^{\frac{1}{2}}\text{days}$)) for each compartment for each selected compound.
[Click here to see definitions of each row \(model compartment\)](#)

The time course data download and simulation parameter data tab and TK summary data tab for example 2.

As with Example 1, we suggest that the user clicks the *Reset Session* button under the *Actions* card if the user wishes to run a new simulation.

Example 3

Let's say we want to run a simulation that outputs rat concentration-time profiles for two days for three compounds: Docusate sodium (CAS: 577-11-7), Phenol (CAS: 108-95-2), and Potassium Benzoate (CAS: 582-25-2). The simulation will be for three oral exposures a day, but the exposures will happen at hours 0, 6, and 12 every day and will be 1 mg/kg each at hours 0 and 6 and 0.2 mg/kg at hour 12. We will use the 1-compartment model and include compounds with in silico generated parameters in place of missing in vitro data. We would like the output at every hour during the simulation.

General Parameters Tab

As with the previous examples, we select *Concentration-time profiles* as the desired output under the *Output* card. As with example 2, we select *Rat* species for the first drop down under the *Species* card. Now, since we are okay with substituting human in vitro data for compounds missing rat in vitro data, we select *Yes* for the second drop down menu under the *Species* card. The first tab should now look like the page below.

INSTRUCTIONS <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>Vignettes (ToCS tutorials)</p> <p>Report ToCS issues/suggestions</p> <p>httk publication</p> <p>httk CRAN webpage</p>	OUTPUT <p>Select the desired output.</p> <p>Concentration-time profiles</p>	SPECIES <p>Select the species to analyze.</p> <p>Rat</p> <p>Do you want to use human in vitro data if in vitro data for the selected species is missing?</p> <p>Yes</p>
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The completed general parameters tab for example 3.

Model Specifications Tab

Under the *Model* card, we select *1compartment* for the first drop down menu. For the second drop down menu, we select *Yes* since we want to include compounds with in silico generated parameters (hepatic clearance, fraction unbound, caco-2) into the compound list. Finally, we enter 2 into the last box under the *Model* card since we want to run the simulation for two days. Under the *Dosing* card, we keep the first two drop downs on their default values. Under the third drop down, we select *Multiple Doses* since we want to give three doses per day. Then, since the doses we want to give are not evenly distributed throughout the day, we select *No* for the resulting fourth drop down menu. Finally, a fifth box appears where we will enter our dosing regime. We enter all of the times (in days) we want to administer the compounds first, then list the amount of compound at each dosing time directly after. Since we want to administer the compounds at hours 0, 6, and 12 each day for two days, the first part of the list we enter is 0, 0.25, 0.5, 1, 1.25, 1.5. Then, the second part of the list is comprised of the dosing amounts, which are 1 mg/kg for the first two doses of the day and then 0.2 mg/kg for the last dose of each day, so we enter 1, 1, 0.2, 1, 1, 0.2 as the second part of the list. The completed *Model Specification* card should appear the same as 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.

Yes, load in silico parameters

Enter the total simulation time (in days).

2

DOSING

Select the administration method of the compound(s).

oral

Select the units of the administered dose(s).

mg/kg

Select the dosing frequency.

Multiple Doses

Are equal doses given evenly across a 24 hour period (i.e. 1 mg/kg BW every 8 hours)?

No

Enter a list of dose amounts (in the specified units) and times (in days) administered. The list must be entered as time1, time2, dose1, dose2, etc. For example, if at 0, 0.5, and 2 days the doses of 1, 3, and 4 mg/kg/BW were given, respectively, enter 0, 0.5, 2, 1, 3, 4 in the box.

0,0.25,0.5,1,1.25,1.5,1,1,0.2,1,1,0.2

The completed model specifications tab for example 3.

Compound Selection Tab

Because we are loading all in silico generated parameters into ToCS, the *Preloaded Compounds* card will take a few moments to load. When it does, we keep the first drop down menu in the *Preloaded Compounds* card on *Choose from all available chemicals* selection and then type in the compound names or CAS numbers for each compound into the second search bar, where we can select all three compounds. The completed *Compound Selection* tab should look like the image below.

The screenshot shows the 'Compound Selection' tab of the ToCS interface. It is divided into three main sections: 'INSTRUCTIONS', 'PRELOADED COMPOUNDS', and 'UPLOADED DATA'.

- INSTRUCTIONS:** A text area with 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." Below this is a link: [Uploaded Physical-Chemical Data File Folder](#).
- PRELOADED COMPOUNDS:** A section titled "Select the types of compounds you want to simulate." It includes a dropdown menu set to "Choose from all available chemicals". Below the dropdown is a text area with instructions: "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." A list box contains two entries:
 - 577-11-7, Docusate sodium | 108-95-2, Phenol
 - 582-25-2, Potassium benzoate
- UPLOADED DATA:** A section titled "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." It features a "Browse..." button and a text input field showing "No file selected".

The completed compound selection tab for example 3.

Note that if under the *General Parameters* tab we selected *No* for using human in vitro data in place of missing rat in vitro data, then docusate sodium and potassium benzoate would not be available for simulation since they do not have rat in vitro data.

Advanced (Optional) Parameters Tab

Since we want to output the solution at every hour, we add a list of output times (in days) to the textbox under the *Output Specification* card on the right of the page. Each time should be separated by a comma, and we can enter times as either whole numbers, decimals, or fractions. Since there are no other customizations we want to make, the completed *Advanced Parameters* tab should look like the image below.

MODEL CONDITIONS

Would you like to change the initial compound amount in each compartment from its default value of 0 (no compound in the compartment when the simulation begins)?

No, keep the default amounts (default)

Select whether to recalculate the chemical concentration blood to plasma ratio from its in vitro or estimated value using the hematocrit, fraction unbound in presence of plasma proteins, and red blood cell partition coefficient.

Do not recalculate (default)

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

MODEL SOLVER

Select the ODE solver method. See R documentation on the 'deSolve' function for method details.

lsoda

Enter the number of time steps per hour for the solver to take.

4

Select the exponent (power of 10) of the relative tolerance for the ODE solver.

Select the exponent (power of 10) of the desired absolute tolerance for the ODE solver.

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 above).

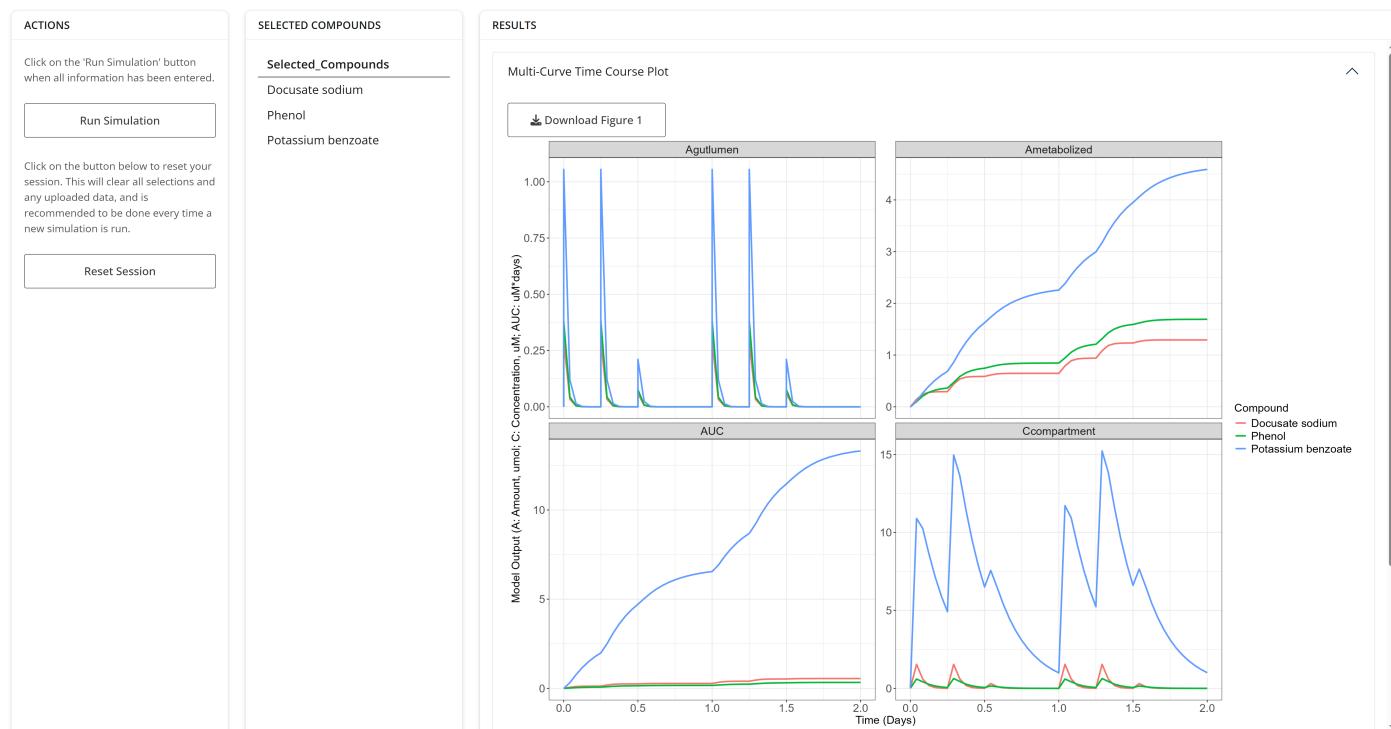
Do not keep Fabs and Fgut at 100% availability (default)

0,1/24,2/24,3/24,4/24,5/24,0.25,7/24,8/24,9/24,10/24,11/

The completed advanced parameters tab for example 3.

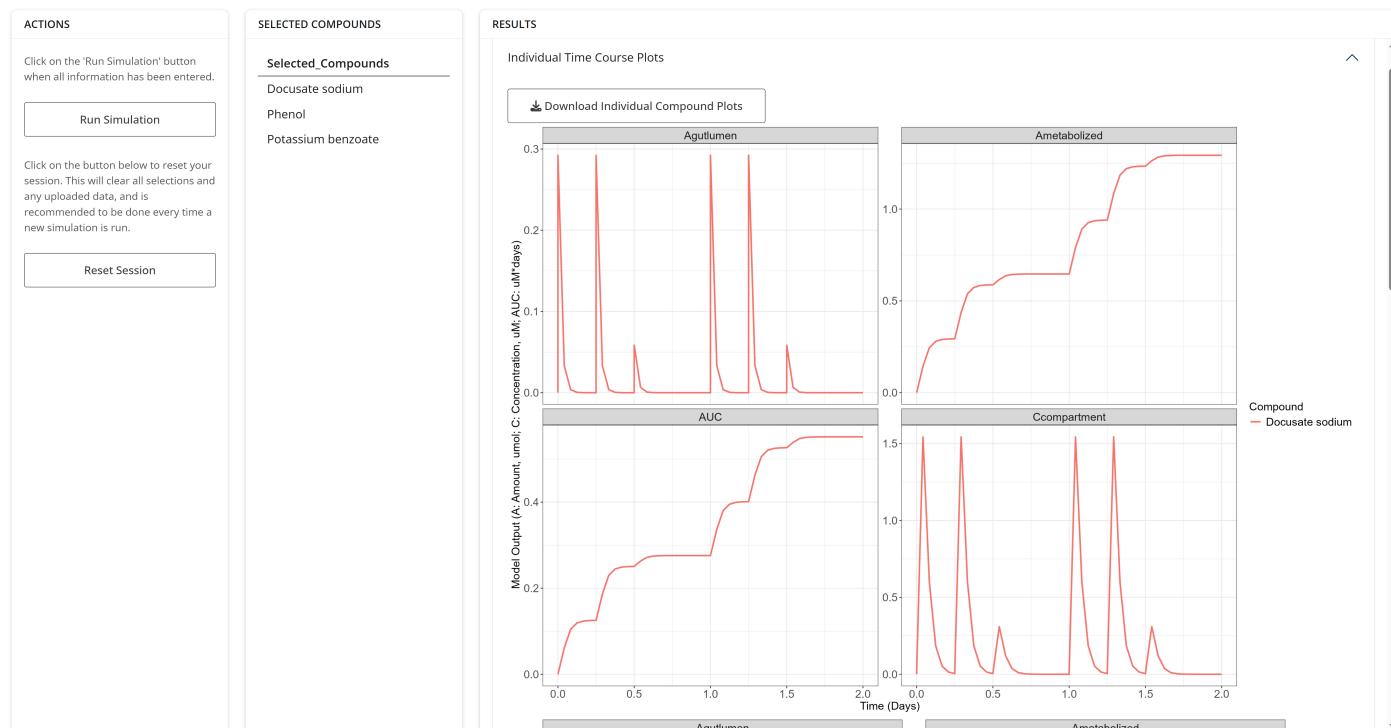
Run Simulation Tab

Now that all previous tabs are completed and the three compounds we want to simulate are shown under the **Selected Compounds** card, so we hit *Run Simulation* under the *Actions* card. When the simulation is complete, it should show the image below. This shows all three simulated compounds overlaying each other for each model compartment with the solution plotted for the times we specified on the previous tab (every hour). The user has the option to download the figure by clicking the *Download Figure 1* button at the top of the drop down menu.



The multi-curve time course plot tab results for example 3.

Below, we see the results for the individual time course plots drop down menu. The user has the option to download all individual plots as a zip file by clicking the *Download Individual Time Course Plots* button.



The individual time course plots tab results for example 3.

The two final tabs shown in the image below give the user the options to download the outputted simulation data, the simulation parameters, and a toxicokinetic summary including Tmax, Cmax, and AUC data for each compound within each model compartment.

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

ACTIONS		SELECTED COMPOUNDS		RESULTS																																				
Click on the 'Run Simulation' button when all information has been entered.		Selected Compounds		Multi-Curve Time Course Plot																																				
Run Simulation		Docusate sodium Phenol Potassium benzoate		Individual Time Course Plots																																				
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.				Time Course Data																																				
Reset Session				Download ADME Time Course Data Download ADME Simulation Parameters																																				
				TK Summary Data																																				
				Download Table 1																																				
		Show 10 entries		Search: _____																																				
				<table border="1"><thead><tr><th></th><th>Tmax.Docusate.sodium</th><th>MaxValue.Docusate.sodium</th><th>AUC.Docusate.sodium</th><th>Tmax.Phenol</th><th>MaxValue.Phenol</th><th>AUC</th></tr></thead><tbody><tr><td>Agutlumen</td><td>0.0001</td><td>0.2923</td><td>0.03365</td><td>0.0001</td><td>0.3825</td><td></td></tr><tr><td>Ccompartment</td><td>0.2917</td><td>1.544</td><td>0.4415</td><td>0.2917</td><td>0.6326</td><td></td></tr><tr><td>Ametabolized</td><td>1.708</td><td>1.293</td><td>1.667</td><td>2</td><td>1.692</td><td></td></tr><tr><td>AUC</td><td>1.75</td><td>0.5517</td><td>0.7111</td><td>1.958</td><td>0.3329</td><td></td></tr></tbody></table>			Tmax.Docusate.sodium	MaxValue.Docusate.sodium	AUC.Docusate.sodium	Tmax.Phenol	MaxValue.Phenol	AUC	Agutlumen	0.0001	0.2923	0.03365	0.0001	0.3825		Ccompartment	0.2917	1.544	0.4415	0.2917	0.6326		Ametabolized	1.708	1.293	1.667	2	1.692		AUC	1.75	0.5517	0.7111	1.958	0.3329	
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				Showing 1 to 4 of 4 entries Previous 1 Next																																				
				Table 1: Table of summary statistics (Tmax - time to maximal concentration, MaxValue - maximal amount (A, umol) or concentration (C, uM), AUC - area under the curve (uM*days)) for each compartment for each selected compound. Click here to see definitions of each row (model compartment)																																				

The time course data and TK summary data tab results for example 3.

As with previous examples, we suggest that the user clicks the *Reset Session* button under the *Actions* card if the user wishes to run a new simulation.