

Impact of *In Vitro* Experimental Variation in Kinetic Parameters on Physiologically Based Kinetic (PBK) Model Simulations

Supplementary Data

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Background information on the *in vitro* CL_{int}, Caco-2 P_{app}, and F_{up} measurements

Background information CL_{int} measurements

Different protocols exist to measure *in vitro* kinetic constants for metabolism. Incubations are most frequently performed with primary hepatocytes or subcellular liver fractions like microsomes or S9 (in the presence of relevant cofactors) (Gouliarmou et al., 2018; Lipscomb and Poet, 2008; Pelkonen and Turpeinen, 2007). Primary hepatocytes are considered the gold standard for performing *in vitro* metabolism studies. Generally, experiments are performed with cryopreserved primary hepatocytes, as these can be stored for a longer period, making them more readily available than freshly prepared hepatocytes. Cryopreserved hepatocytes retain most of the activity of freshly prepared hepatocytes (Lipscomb and Poet, 2008). With a so-called “metabolite formation protocol”, *in vitro* incubations are performed at different substrate concentrations at a fixed incubation time and cell concentration (Fig. S1A). The formation of metabolites, which follows the Michaelis-Menten equation (Seibert and Tracy, 2014), is then measured in these experiments:

$$v = \frac{V_{\max} * [\text{compound}]}{K_m + [\text{compound}]} \quad \text{Eq. S1}$$

In this equation, V_{max} is the maximum velocity (for example μmol/min/10⁶ hepatocytes) and K_m is the Michaelis-Menten constant with the unit μM (Peters, 2012). A key advantage of this approach is that the kinetic constants obtained can be used to describe the formation of metabolites and allow to account for concentration-dependent saturation of the enzymes. A disadvantage of this protocol is that standards of the metabolites are required for quantification. Given that such standards cannot be easily obtained for most compounds, the metabolic conversion of compounds is more frequently measured with a so-called “substrate depletion approach” in which the disappearance of a compound is measured over time to derive CL_{int} based on the slope of the substrate depletion curve (Jones and Houston, 2004). One of the most critical aspects of substrate depletion experiments is that the substrate concentration should be well below the K_m (linear region), as only then the rate, v, can be simplified as depicted in Equation 2 (Seibert and Tracy, 2014).

$$v = \frac{V_{\max}}{K_m} * [\text{compound}] = \text{CL}_{\text{int}} * [\text{compound}] \quad \text{Eq. S2}$$

The obtained CL_{int} values can therefore only be used in situations where no saturable metabolism is to be expected. This can be explored with *in vitro* range finding experiments at different concentrations to determine at which concentrations saturation of metabolism occurs (Nichols et al., 2018; Sjögren et al., 2012). First estimates of the internal concentrations with a PBK model can be used to determine if these saturable conditions are likely to be reached in the liver. Other aspects that need to be considered when performing *in vitro* metabolic clearance studies are, for example, the protein amount in the incubation mixture, whether or not serum is added to the incubation, number of time points and sampling schedule, the percentage of test item consumption at the end of the incubation, and aspects related to the analytical techniques that are used to analyse the sample (Gouliarmou et al., 2018; Louisse et al., 2020). In addition, it is important to include positive controls (marker substrates for different metabolic enzymes) in the experimental setup to check the proper performance of the test system (Hernandez-Jerez et al., 2021).

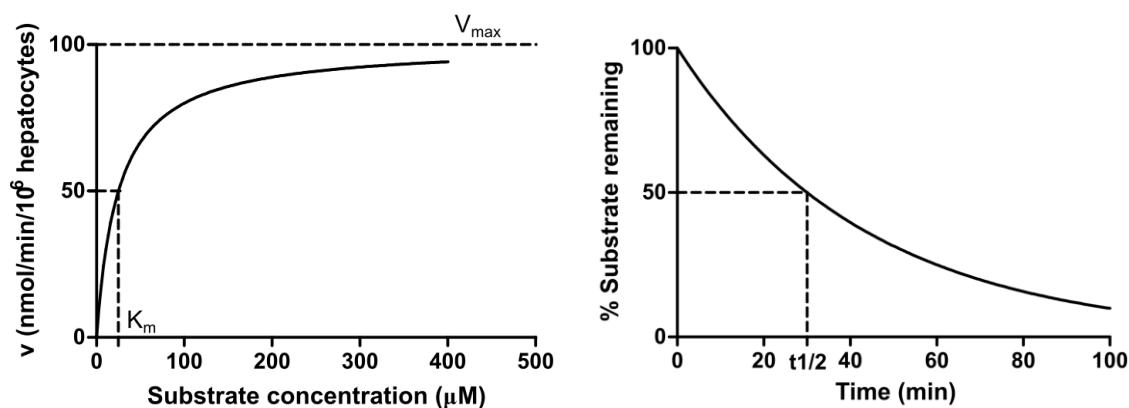


Fig. S1: Examples of A) Michaelis-Menten kinetics with a K_m of 25 μM and a V_{max} of 100 nmol/min/ 10^6 hepatocytes, and B) a metabolic clearance study with a $t_{1/2}$ of 30 min and a CL_{int} of $\ln(2)/30 = 0.02 \text{ mL/min}/10^6$ hepatocytes when performed in an incubation that contains 10^6 hepatocytes per mL

Background information on Caco-2 P_{app} measurements

The Caco-2 cellular model of intestinal absorption is one of the most frequently used *in vitro* test system to study the rate of transport of compounds across the intestinal cell layer. Although Caco-2 cells are derived from a human colon carcinoma, the cells mimic the epithelial barrier of the small intestine when cultured in a monolayer (Hubatsch et al., 2007). For *in vitro* P_{app} measurements, the cells are grown in a so-called Transwell system, in which the cells are seeded on a permeable filter insert and are cultured for about 21 days to form a layer of differentiated cells. To measure the cellular transport of a compound, the cell culture medium at the apical compartment of the Transwell is replaced by a transport buffer in which the compound is dissolved, and the cell culture medium at the basolateral compartment is replaced by the transport buffer, often containing bovine serum albumin to mimic the protein content of the blood compartment (Hubatsch et al., 2007). A critical aspect of P_{app} measurements is that the experiments are performed under a concentration gradient, otherwise diffusion cannot take place. This means that the time-range in which the absorption studies are performed needs to be optimized to make sure that less than 10% of the compound is diffused to the basolateral compartment (also called sink-conditions) (Usansky and Sinko, 2005). Such sink conditions provide the best representation of physiological conditions, as a concentration gradient between the gut lumen and the plasma will exist *in vivo* due to distribution of the chemical in the body after absorption. In addition, it should be noted that Caco-2 experimental results often vary between labs and with batches of cells. Therefore, a range of reference substrates should be included in the experimental setup to normalize the results. A final important experimental aspect that can affect the P_{app} measurement is the pH gradient that is applied between the apical and basolateral compartment. A pH gradient of 6.5-7.4 provides the best representation of the physiological conditions in the intestinal lumen and blood (Neuhoff et al., 2003).

Background information on F_{up} measurements

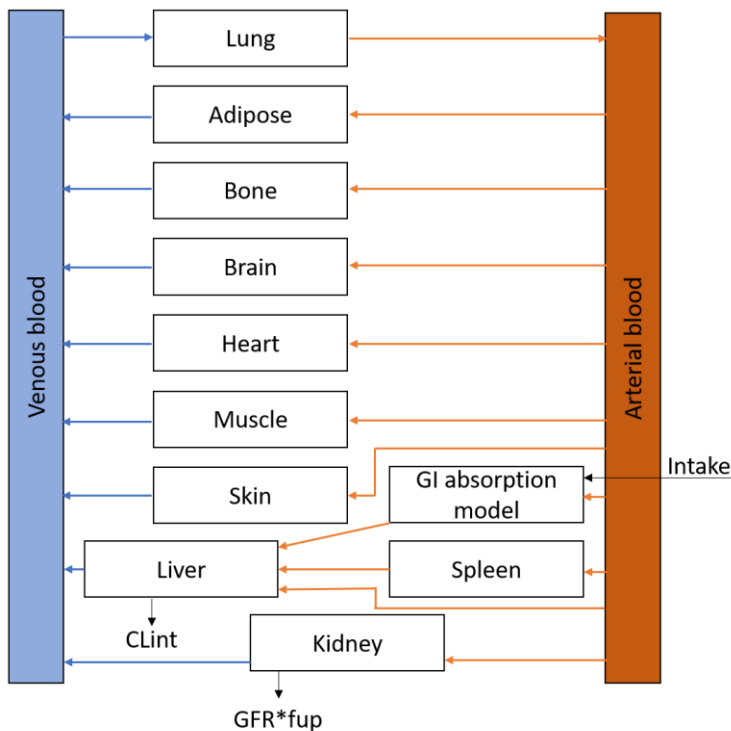
Various methods have been developed to measure F_{up} , the equilibrium dialysis test system being the most commonly applied. For these experiments, so-called equilibrium dialysis devices are used, which consist of a base plate and different dialysis inserts. Each of the dialysis inserts consists of two chambers separated by a dialysis membrane. The human plasma, generally containing 2 to 5 μM of the substrate, is added to one chamber and phosphate-buffered saline (PBS) to the other (Ryu et al., 2021). The concentrations in the two chambers are monitored until an equilibrium is reached. The equilibrium dialysis technique particularly poses challenges with measuring the fraction unbound for highly protein-bound compounds. For these compounds the levels in the receiving PBS chamber may be close to the limit of detection, hampering derivation of F_{up} values. In addition, there is a higher chance for non-specific binding for these compounds. For highly bound compounds, modified equilibrium dialysis has therefore been proposed, including bidirectional equilibrium dialysis, dilution methods, and pre-saturation methods (Ferguson et al., 2019; Wambaugh et al., 2019).

References

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OECD harmonized template description of the PBK model

Category	Characteristic
Scope and purpose of the model	<p><u>Model purpose</u>: generic model code to simulate plasma and tissue concentrations of chemicals (parent chemicals, not metabolites) based on a minimal set of (<i>in vitro</i>- and/or <i>in silico</i>-derived) chemical-specific input parameters (metabolic clearance, blood:plasma partition coefficients, fraction unbound plasma and blood:plasma ratio)</p> <p><u>Species</u>: human.</p> <p><u>Age, life stage(s), sex, exposure window(s)</u>: adult 70 kg (human), not gender specific.</p> <p><u>Exposure route(s) and dose metric(s)</u>: IV and oral (mg/kg bw)</p> <p><u>Target organs and tissues</u>: lung, adipose, bone, brain, heart, muscle, skin, liver, kidney, gut, spleen, venous and arterial blood.</p>
Model structure and mathematical description	<p>13 perfusion-limited compartments</p> <p>Differential equations</p> <p>Perfusion-limited</p> <p>Mass balance equations given</p>
	 <p>Absorption: First order rate constant (Jones and Rowland-Yeo (2013))</p> <p>Distribution: Homogenous and blood-flow limited distribution was assumed in each compartment.</p> <p>Metabolism: Linear with dose (no saturation included)</p> <p>Excretion: Urinary excretion is included in the kidney compartment as glomerular filtration rate times the free venous plasma concentration.</p>
Computer implementation	<p>Model implemented in R</p> <p>Model codes and syntax available</p>
Parameter estimation and analysis	<p>Anatomical and physiological parameter values as reported by Jones and Rowland-Yeo (2013).</p> <p>Partition coefficients Rodgers and Rowland (2006).</p> <p>Intrinsic hepatic clearance (<i>in vitro</i> intrinsic hepatic clearance data are scaled to the <i>in vivo</i> situation).</p> <p>F_{up} from <i>in vitro</i> experiments.</p> <p>Intestinal uptake scaled from <i>in vitro</i> Caco-2 apparent permeability data as described by Punt et al. (2021).</p> <p>Global sensitivity analysis with Rvis (https://github.com/GMPtk/RVis/releases, v0.15, using R 4.1.1)</p>

Model calibration and validation	<p>Calibration: <i>In vitro</i> and <i>in silico</i> input data are used for the parameterization to make an estimation of the <i>in vivo</i> toxicokinetics. No calibration step is therefore needed.</p> <p>Validation: Generally, adequate estimations of <i>in vivo</i> kinetic parameters (particularly C_{max}) can be made with the model for chemicals that are rapidly absorbed and when liver metabolism is the main clearance route. However, this is not the case for chemicals that will largely depend on, for example, extrahepatic metabolism and/or active-transporter-mediated kinetics. Without data to evaluate model performance, one should therefore be cautious when applying the model for different chemicals. Predictions need to be evaluated on a case-by-case basis.</p>
Model documentation	<p>Peer reviewed model</p> <p>Publicly available model</p>

Evaluation of the PBPK model according to WHO criteria

The goal of the model of Jones and Rowland-Yeo (2013) is to make first-tier estimates of expected plasma and/or tissue concentrations making use of a minimal set of chemical-specific input data, i.e., intrinsic hepatic clearance, partitioning into tissues, the fraction unbound in plasma, and the blood:plasma ratio. The model structure contains the major body compartments (lung, adipose, bone, brain, heart, muscle, skin, liver, kidney, gut, spleen, venous and arterial blood). The original model of Jones and Rowland-Yeo (2013) was adopted by Punt et al. (2021) for use in an online platform (www.qivivetools.wur.nl) and modified with respect to the following points:

- Conversion of the model code from Berkeley Madonna to R, solving the differential equations with the R deSolve package.
- $C_{liverfree}$ defined as $C_{liver}/K_{pi} \cdot F_{up}$ instead of $C_{liver} \cdot F_{up}$.
- Fraction absorbed (F) accounted for in the initial setting of the dose rather than the differential equation for oral absorption (the latter would be the rate of absorption and not the fraction that is absorbed).
- Renal clearance (urinary excretion) simulated as GFR times the free plasma concentration. (Within the model of Jones and Rowland-Yeo (2013), renal clearance is described as $CL_{renal} \cdot C_{kidneyfree}$ and it is not specified whether this renal clearance corresponds to urinary excretion or metabolic clearance.)
- Conversion of plasma concentration to nM as output of the model.
- K_{pre} (tissue:partition coefficient rest body) set equal to the muscle partition coefficient.
- Mass balance equations added.

References

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To import the PBK model, follow steps 1 to 13. These steps are only required ones.

1. Open Rvis and click on import R

2. Select the model code with Browse

4. Click inspect

3. Select the model code

The screenshot shows the Rvis software interface. The main window has a menu bar with 'SIMULATION', 'IMPORT R', 'IMPORT MCSIM', 'LIBRARY', and 'ACAT'. Below the menu bar, there is a 'Browse...' button and a text prompt '(Select a .R file)'. The 'Import Simulation' dialog box is open, showing a file explorer view. The 'File name' field contains 'humanGenericPBK_Caffeine'. The 'File type' is set to 'R Files'. The 'Open' button is highlighted. The dialog box also shows a list of files in the background, including 'humanGenericPBK_Caffeine', 'humanGenericPBK_Diazepam', 'humanGenericPBK_Diltiazem', 'humanGenericPBK_Midazolam', 'humanGenericPBK_Quinidine', and 'humanGenericPBK_Verapamil'. The 'Inspect' button is visible in the top right corner of the main window.

USE EXECUTIVE FUNCTION

To use this import option, your code must provide a function vector or list of parameters, and whose return type is a matrix. Before proceeding, identify both the function and the list object parameters.

Exec fn: ?

Param obj: ?

Output tbl: ?

Indep var: ?

Import...

Import...

Ready

46.7 fps

RVIS

SIMULATION
IMPORT R
IMPORT MCSIM
LIBRARY
ACAT

Browse...
C:\Users\anspu\OneDrive - WageningenUR\Models\Rvis\Gargas1\humanGenericPBK_Caffeine.R
Inspect

Globals: 2 unary fn(s) 0 scalar(s) 1 scalar set(s) 1 data set(s)

USE EXECUTIVE FUNCTION

To use this import option, your code must provide a function whose single argument is a vector or list of parameters, and whose return type is a matrix, a dataframe, or a list. Before proceeding, identify both the function and the list object containing the parameters.

?

Exec fn: ?

Param obj: ?

Output tbl: ?

Indep var: ?

Import...

5. Click Select

Select...

USE TEMPLATE

To execute your simulation, your code will be sourced by RVis each time. To vary input parameters, RVis will edit your code and re-write the R file before sourcing it. In the following dialog, you will enter assignments and parameter values.

SELECT EXECUTIVE FUNCTION

Executive function

run_model

Parameters

parameters

OK

Cancel

6. select "run_model" here

7. select "parameters" here

8. click "OK"

Import...

Rvis

SIMULATION
IMPORT R
IMPORT MCSIM
LIBRARY
ACAT

Browse...
C:\Users\anspu\OneDrive - WageningenUR

USE EXECUTIVE FUNCTION

To use this import option, your code must provide a function that returns a vector or list of parameters, and whose return type is a matrix. Before proceeding, identify both the function and the list of parameters.

Exec fn: run_model
Param obj: parameters
Output tbl: 38 column(s)
Indep var: time

IMPORT SIMULATION (EXEC)

output <- run_model(parameters)

PARAMETERS
OUTPUT
LIBRARY

Use?	Name	Value	Unit	Description	Edit
<input checked="" type="checkbox"/>	BP	1			
<input checked="" type="checkbox"/>	BW	70			
<input checked="" type="checkbox"/>	CINT	0.1		Exposure Definitions; Communication interval	
<input checked="" type="checkbox"/>	CLint	1.595			
<input checked="" type="checkbox"/>	CLrenal	6.7			
<input checked="" type="checkbox"/>	FQad	0.05			
<input checked="" type="checkbox"/>	FQbo	0.05			

Use All
Use None

Import
Cancel

9. click "Import"

10. click "Use All" to select all parameters

11. go to the "OUTPUT" tab

RVIS

SIMULATION
IMPORT R
IMPORT MCSIM
LIBRARY
ACAT

Browse...
C:\Users\anspu\OneDrive - WageningenUR

USE EXECUTIVE FUNCTION

To use this import option, your code must provide a function vector or list of parameters, and whose return type is a matrix. Before proceeding, identify both the function and the list parameters.

Exec fn: run_model
Param obj: parameters
Output tbl: 38 columns
Indep var: time

IMPORT SIMULATION (EXEC)

output <- run_model(parameters)

PARAMETERS
OUTPUT
LIBRARY

Independent Variable

time Unit: ? Description: ?

Use?	Name	Unit	Description	Edit
<input checked="" type="checkbox"/>	Cplasmavenous.AVE			
<input type="checkbox"/>	Cskin.Ask			
<input type="checkbox"/>	Espleen.Asp			
<input type="checkbox"/>	Evenous.Ave			

Use All
Use None

Import
Cancel

12. Click on USE? in front of Cplasmavenous.AVE and AUC. These output parameters will be plotted

13. Click on "Import"

Import...

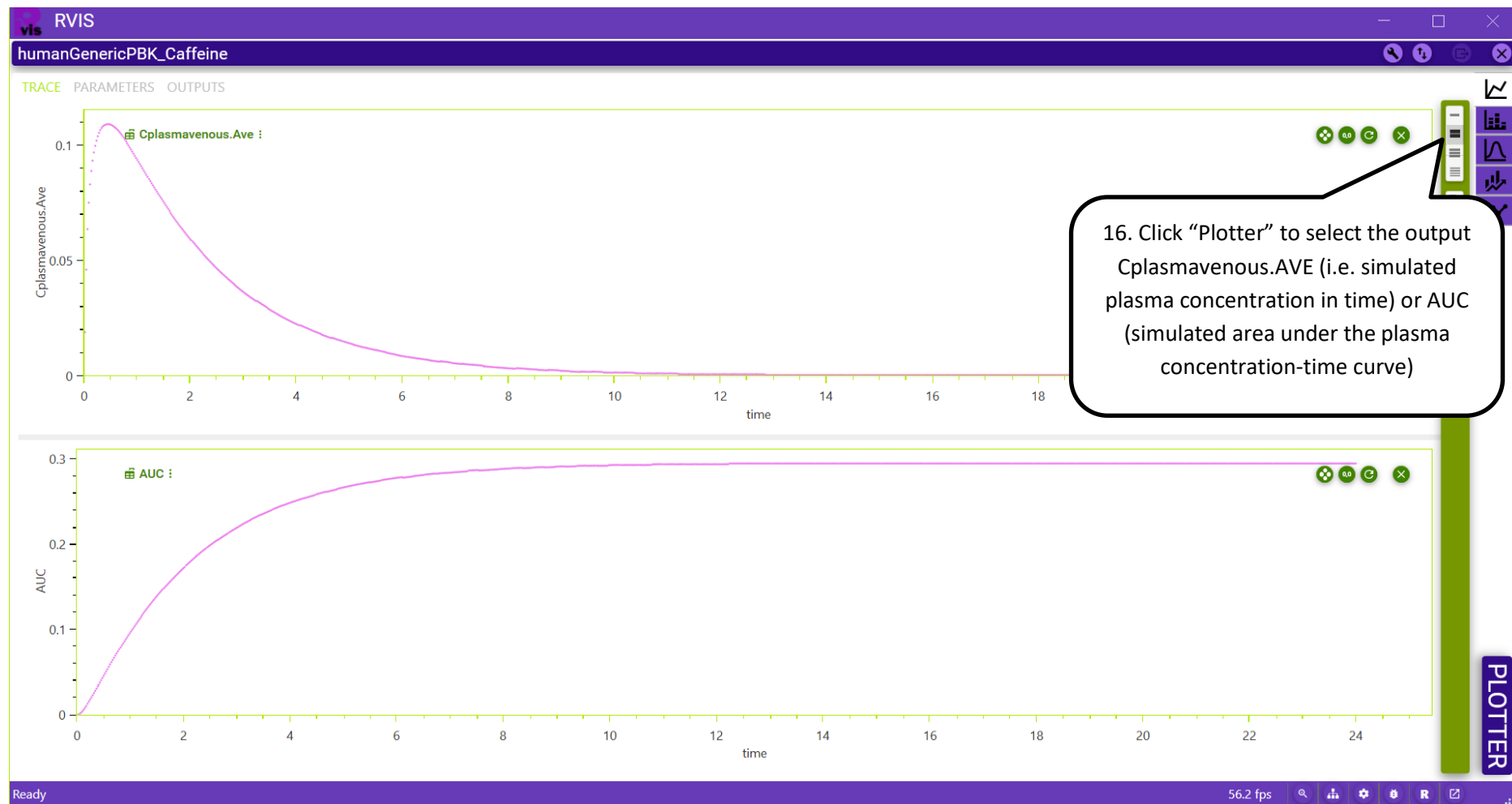
Ready
45.5 fps

To run the model follow steps 14 to 16 to run the model

The screenshot shows the RVis software interface. At the top, there is a purple header bar with the 'RVis' logo and navigation tabs: 'SIMULATION', 'IMPORT R', 'IMPORT MCSIM', 'LIBRARY', and 'ACAT'. The 'LIBRARY' tab is active, displaying a table of imported models. A callout box labeled '14. Select one of the imported models' points to the first row of the table. To the right of the table, there are two circular buttons: a play button (labeled '15. Click "Run"') and a folder icon. The right side of the interface shows the version 'RVis v0.15' and the text 'using R v4.1.1'. The bottom status bar indicates 'Ready' and '50.3 fps'.

Name	Description
humanGenericPBK_Caffeine	
humanGenericPBK_Diazepam	
humanGenericPBK_Diltiazem	
humanGenericPBK_Midazolam	
humanGenericPBK_Quinidine	
humanGenericPBK_Verapamil	

Library location: ~/RVisSim



Follow steps 17 to 27 to perform the sensitivity analysis

vis RVis

humanGenericPBK_Caffeine

PARAMETERS DESIGN SAVED DATA

Select Parameters

☐ BP ☐ BW ☐ CINT ☒ CLint ☐ CLrenal ☐ FQad ☐ FQbo ☐ FQbr ☐ FQgu

☐ FQh ☐ FQhe ☐ FQki ☐ FQlu ☐ FQmu ☐ FQre ☐ FQsk ☐ FQsp ☐ FQte

☐ fuhep ☒ fup ☐ FVad ☐ FVar ☐ FVbo ☐ FVbr ☐ FVfu ☐ FVhe ☐ FVki

☐ FVli ☐ FVlu ☐ FVmu ☐ FVpl ☐ FVrb ☐ FVre ☐ FVsk ☐ FVte

☐ FVve ☐ Kpad ☐ Kpbo ☐ Kpbr ☐ Kpgu ☐ Kphe ☐ Kpki ☐ Kpli ☐ Kplu

☐ Kpmu ☐ Kpre ☐ Kpsk ☐ Kpsp ☐ Kpte ☐ MW ☐ ODOSE ☒ PappAB

☐ POINTS ☐ QC ☐ SF ☐ TSTOP

Parameter QF Distribution

CLint	CLint ~ $N(\mu = 1.595, \sigma^2 = 0.09)$ [1, 2.2] <input checked="" type="checkbox"/>
fup	fup ~ $N(\mu = 0.968, \sigma^2 = 0.04)$ [0.57, 1.4] <input checked="" type="checkbox"/>
PappAB	PappAB ~ $N(\mu = 37.27, \sigma^2 = 49)$ [23, 51] <input checked="" type="checkbox"/>

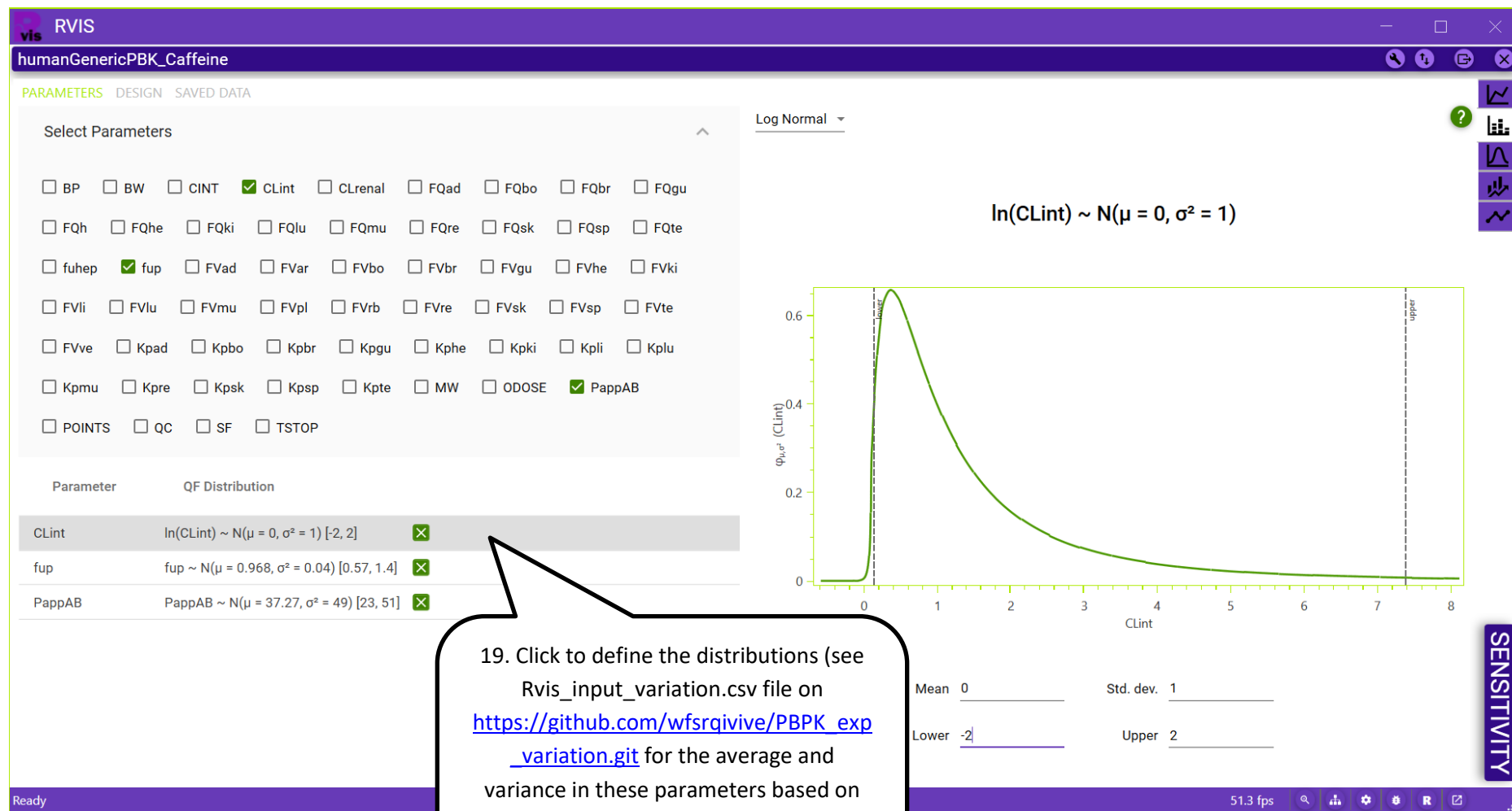
Select a parameter to continue

17. Select the "Sensitivity" tab

18. Select "CL_{int}", "f_{up}" and "P_{appAB}" as parameters for the sensitivity analysis

SENSITIVITY

Ready 38.7 fps



RVIS
humanGenericPBK_Caffeine

PARAMETERS DESIGN MEASURES EFFECTS SAVED DATA

FACTORS/DISTRIBUTIONS

$\ln(\text{CLint}) \sim N(\mu = 0, \sigma^2 = 1) [-2, 2]$
 $\text{fup} \sim U(a = 0.6, b = 1)$
 $\ln(\text{PappAB}) \sim N(\mu = 3.6, \sigma^2 = 0.0001) [3.58, 3.62]$

INVARIANTS

NONE CONFIGURED

METHOD

☐ Morris ☒ e-FAST

No. of samples: 100

Create Design

DESIGN

Created on 2021-11-14 22:49:33

Unload Design

ACQUIRE OUTPUTS

Start

0 / 300

SAMPLES

CLint	fup	PappAB	Acquired?
1	0.8	36.59823444367799	No
1.8404916365292936	0.808	36.61575813803211	No
5.6390766478732	0.8160000000000001	36.63333052317029	No

Share

View Error

☐ Show issues only

SENSITIVITY

Ready

56.4 fps

20. Click on the "Design" tab

21. Select "e-FAST" and 100 samples

22. Click "Create Design"

23. Click "Start"

