

Introduction to Dallphin-AtoM (ver. 0.8.6)

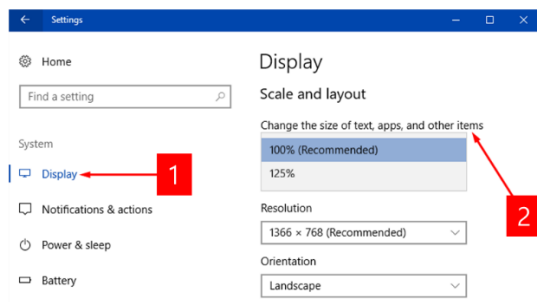
If you are using a laptop or an internal or external monitor with Ultra High Definition (4k UHD) using a higher resolution, you might experience the following problems in Dallphin.

- Elements are too small or large compared to the rest of the desktop.
- Elements such as icons, toolbars, text, and dialog boxes appear to be fuzzy or disoriented.
- Blurry text appears in Dallphin interface.

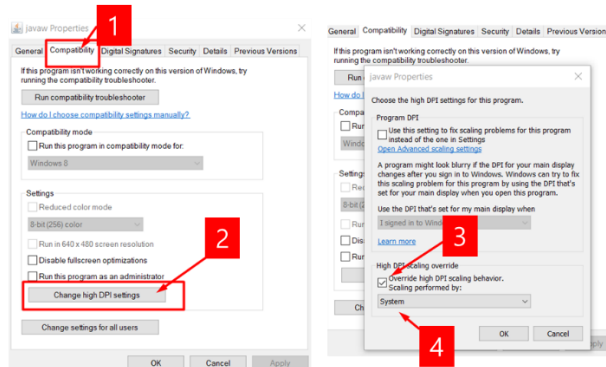
You can solve these problems by applying one of the following solutions.

1. Change the size of apps: You can right-click on the Desktop (background image) > Display Setting > Change the size of apps, text, and other items on the screen. (100%) (Figure 1A)
2. Change the Java compatibility properties of Javaw.exe: You can find javaw.exe in C:\WDALL-PhinWjreWbin in the Explorer and follow these actions: Right click > Properties> Compatibility tab > Change high dpi settings > Click override system settings. (Figure 1B)

A



B



Release note (0.8.5 → 0.8.6)

1. In the Absorption tab, the reference Caco-2 Papp values of propranolol and atenolol that are used for the calibration of the user-input Papp were replaced with those obtained from the method of Usansky's report (J Pharmacol Exp Ther. 2005; 314: 391-399): but, they are not visible to users.
2. The Fa_Caco calculation method was replaced with that used in the Usansky's report (J Pharmacol Exp Ther. 2005; 314: 391-399) to avoid suspected overestimation.
3. Resolution of simulated concentration plots was improved.
4. Bugs in the automatic parameter calculation were fixed.

1. What is Dallphin-AtoM?

- Dallphin is the acronym of the phrase 'Drugs with **ALL**ometry and **PH**ysiology **IN**side' and AtoM is '**A**nimal to hu**M**an'.
- It is a PBPK software that predicts human PK parameters and plasma drug concentrations based on many published methods using physicochemical properties, in vitro and animal PK data.
- This beta version is for Windows (x64). (Mac version is to be distributed in 2019.)

2. Why Dallphin-AtoM?

- There are several commercial and free PBPK software packages already available for human PK prediction.
- However, we felt needs for something that does not request overly many parameters and that clarifies all of the methods and/or references used to predict human PK to the researchers using the software.
- Also, less restriction in the accessibility, right to use or affordability for both of the academia and industry was what we've sought for.

3. Right to use Dallphin-AtoM

- Dallphin-AtoM (Dallphin, for simplicity) 0.8.6 is distributed to beta testers for test use only.
- Any suggestion, comments or bug reports via email (yimds@catholic.ac.kr) are welcomed.

4. Who developed Dallphin-AtoM?

- Dallphin 0.8.6 was developed by researchers of PIPET (Pharmacometrics Institute for Practical Education and Training) of the College of Medicine, the Catholic University of Korea for the education of students and researchers.
- Dallphin 0.8.6 was developed as a part of the EDISON (EDucation-research Integration through Simulation On the Net) Program of the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (grant number: 2016M3C1A6936614).

5. Disclaimer

- All of the predictions by Dallphin are calculated based on equations published in research articles (peer-reviewed journals) and textbooks on PK, PBPK, and allometry. The accuracy, adequacy, validity, reliability, or completeness of any information provided by Dallphin is dependent on the references that were used in Dallphin. Under no circumstances shall the developers of Dallphin have any liability to the users for any loss or damage of any kind incurred as a result of the use of Dallphin or reliance on any information provided by Dallphin.
- The user may not modify contents of the Dallphin, nor distribute the modified installation files.

6. What this manual tells

- To predict drug exposure in human, we need to know four major PK parameters - K_a , V_d (V_c , V_p , and Q), CL and F if the drug follows first-order kinetics.
- This manual briefly introduces the principles used in the prediction of each of the 4 PK parameters based on in vitro or animal PK data together with corresponding references.
- You may find more detailed theories and equations used in Dallphin in two lecture slides (pdf documents) that may be downloaded by clicking on the "About" tab of Dallphin.

Brief manual for Dallphin-AtoM (ver 0.8.6)



- When Dallphin is launched in your computer, you'll see seven tabs. From the leftmost one, you should enter your laboratory results requested in each tabpane.
- When the entering job is done from the 'Basic info' to the 'Elimination' tab, you may click the 'Final Parameters' tab to see all of the predicted human PK parameters.
- Lastly, you will observe the predicted plasma concentrations at the "Concentration Prediction" tabpane.

Dallphin-AtoM(ver 0.8.5)

Basic Info Absorption Distribution Elimination Final Parameters Concentration Prediction About

Physicochemical Properties

Neutral

Log P 0

pKa 0

Log D7.4 Prediction 0

☒ PSA 0 Å²

Plasma Protein Binding

☒ fu_Rat 0

☒ fu_Dog 0

fu_Hum 0

Cb/Cp 1.0

1. Basic info Tab

- In the Basic info tabpane, physicochemical properties, blood-to-plasma concentration ratio,

and plasma protein binding of the user's candidate molecule (test drug) are input.

- Log D may be directly inputted by the user or calculated from log P and pKa values.
- The physicochemical property inputs are used at the calculation of unbound fraction in the microsome or hepatocytes at the 'Elimination' tab. The unbound fraction is then used to predict hepatic CL, although it does not appear as final parameters.
- When PSA (polar surface area) value is input, Fa calculated using PSA [1] is shown at the Absorption tabpane as Fa_PSA.

2. Absorption Tab

1) Caco-2 permeability and Fa

- Caco-2 permeability of the test drug should be input as Papp. Those from reference standard drugs (propranolol and/or atenolol) are also recommended to input to calibrate the test drug's Papp in a bid to minimize the inter-laboratory or inter-occasional differences in Papp assay data.
 - In Dallphin-AtoM, the calibration method is based on the Fa predicted using the equation proposed by Usansky et al. [2] The reference Papp values of propranolol (21.4×10^{-6} cm/sec) and atenolol (0.37×10^{-6} cm/sec) are those expected to give the predicted Fa identical to known Fa values of propranolol (90%) and atenolol (56%), respectively. The ratios of user-input Papp versus the ideal Papp of the two reference drugs are used to calibrate the Papp of the test drug.
- The Peff (permeability in human duodenum) is calculated from the Papp (or corrected Papp if Papp of reference drugs are available) which is in vitro data [3]. (The Peff is provided for reference, and it not used for parameter prediction.)
- Caco-2 cell-based Fa (Fa_Caco, Fa: fraction absorbed, $F = Fa \times F_g \times F_h$) was then calculated using equations on the relationship between Fa, Papp, and small intestine surface area. [2] The Fg was fixed to 1 in Dallphin. (The Fa_Caco calculation method was changed in the version 0.8.6)
- MDCK-II cell permeability can be input or predicted using Caco-2 Papp to estimate passive CL that may be used when the microsomal method is chosen for the calculation of hepatic CL (Elimination tabpane).

- The F_a calculated herein is given as F_{a_Caco} in the bottom of the pane.

2) MDCK permeability

- MDCK-II cell permeability may be input here when available. It is used for the calculation of microsome-data based hepatic CL (Elimination tabpane).

3) first-order absorption rate constant: k_a

- We used an equation to predict human k_a that is calculated from Caco-2 permeability data, intestinal surface area and V_c (central compartment V_d) [2].
- In a 1-compartment model, to predict the k_a value, we assume the V_d (V_{ss}) as V_c . This may cause some discrepancy in k_a prediction. Thus, we highly recommend you to use a 2-compartment model.

**The first-order oral absorption model has been criticized as physiologically unreliable and sophisticated models such as ADAM or ACAT have been proposed. However, to use those models properly, the user should input detailed information on the metabolic pathways and metabolic ratios of the molecule, which is not commonly practiced in preclinical development phases unless the company has an internal policy of intense metabolic profiling before clinical development. Thus, Dallphin employed the conventional parameter k_a , assuming first-order absorption for simplicity. (Though this may not be sophisticated, seems cost-effective in preclinical to clinical prediction.)*

3. Distribution tab

- Both of the allometric method (proportional to body weight) using animal PK data and the PBPK model-based method using physicochemical properties and physiological values are available to predict human V_d in Dallphin-AtoM. The user should opt for one in the two.

1) when using the allometric method

- Compartmental PK parameters on the distribution (V_c , V_p , and Q , the inter-compartmental CL) obtained from i.v. PK study in more than two animal species (mouse, rat, dog, monkey) is a necessity to use the allometric method.
- Allometric exponents for V 's were fixed to 1 and that for Q was fixed to 0.75. (Estimation of the exponents is to be provided in the future version.)

- The user may choose either one- or two-compartment models, and the number of compartments should be the same for all of the animal species used. (The number of distribution compartments ≥ 3 is not acceptable.)

(1) In the case of 2-compartment model:

- V_c and V_p in each species are to be input by users and V_{ss} is automatically filled up ($V_{ss} = V_p + V_c$).
- Dallphin calculates human V_{ss} and V_c allometrically with the exponent to body weight fixed to 1.
- Although there is no report on the allometric estimation of V_c , we assumed that both of V_c and V_{ss} are proportional to body weights regardless of species.

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Basic Info | Absorption | Distribution | Elimination | Final Parameters | Concentration Prediction | About

Animal (in vivo) I.V. PK data in ≥ 2 species available? ☒ Yes ☐ No

Allometric approach

Enter distribution data in animals Two-Compartment Model

Species	Weight	Q	V_c	V_p	V_{ss}
<input checked="" type="checkbox"/> Mouse	20 g	0.8 L/hr/kg	0.5 L/kg	1.0 L/kg	1.5 L/kg
<input checked="" type="checkbox"/> Rat	200 g	0.8 L/hr/kg	0.5 L/kg	1.0 L/kg	1.5 L/kg
<input checked="" type="checkbox"/> Monkey	3.0 kg	0.8 L/hr/kg	0.5 L/kg	1.0 L/kg	1.5 L/kg
<input checked="" type="checkbox"/> Dog	10.0 kg	0.8 L/hr/kg	0.5 L/kg	1.0 L/kg	1.5 L/kg

Prediction

Allometric Scaling Result

Human WT : 70kg Q : 0.47 L/hr/kg V_c : 0.5 L/kg V_p : 1.0 L/kg V_{ss} : 1.5 L/kg

VSS (unit:L)

$H=1.5, r^2=1$

VC (unit:L)

$H=0.5, r^2=1$

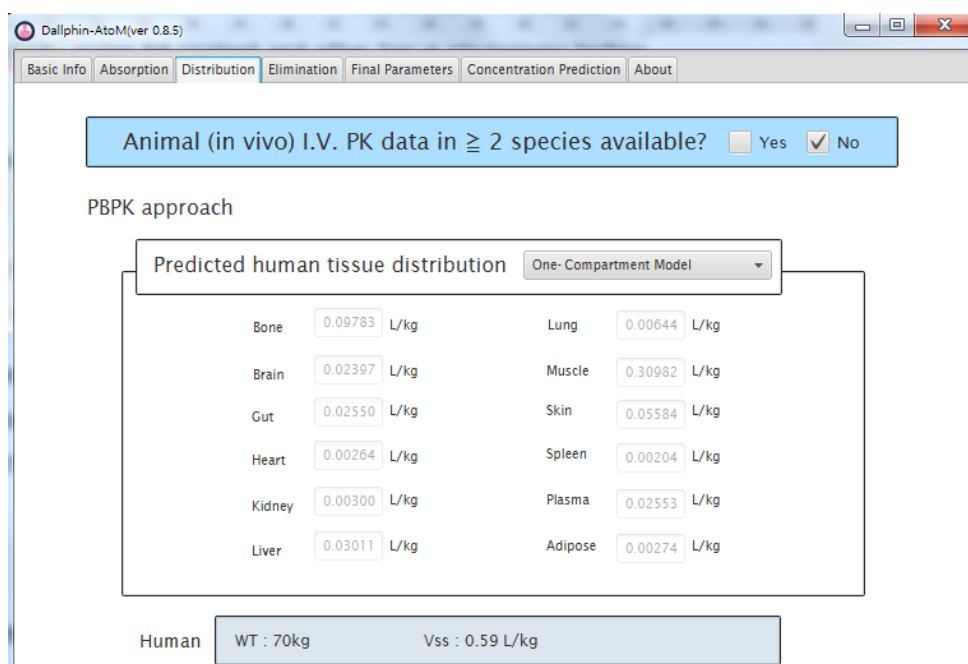
(2) In the case of 1-compartment model:

- The user may also opt for "1-compartment" model instead of "2-compartment model". (But, using the 2-compartment model strongly recommended!)

- Occasionally, the i.v. PK data may be better fitted by a 1-compartment model according to the PK sampling scheme, infusion time, or else. Even when the i.v. PK profiles in animals seem to follow multi-compartment models, software for compartmental analysis may not be available to the user. Then, the user may input Vss obtained from non-compartmental analysis by selecting the "1-compartment" model.
- Vp or Q are not requested.

2) when using the PBPK method

- If the animal in vivo PK data is not available, Vss may be predicted from the PBPK-based method which uses drug lipophilicity, protein binding (Basic info tab), organ fat content and other tissue physiologic factors. [4-6]
- All of the physiological variables from the references are used in Dallphin AtoM as built-in equations to calculate Vss.
- When using the PBPK method, only Vss is given to the user (without presenting Vc and Vp separately) as if the drug follows a 1-compartment model.
- Although the Vc is also calculated using PBPK information for well-perfused organs, it is only internally used to calculate the absorption rate constant (k_a) and Fa_{Caco} . (Because there is no reliable PBPK method to predict Q in human, a 2-compartment model is not used in the PBPK method even though the Vc is obtained.)



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Basic Info Absorption Distribution Elimination Final Parameters Concentration Prediction About

Animal (in vivo) I.V. PK data in ≥ 2 species available? ☐ Yes ☒ No

PBPK approach

Predicted human tissue distribution One-Compartment Model

Bone	0.09783 L/kg	Lung	0.00644 L/kg
Brain	0.02397 L/kg	Muscle	0.30982 L/kg
Gut	0.02550 L/kg	Skin	0.05584 L/kg
Heart	0.00264 L/kg	Spleen	0.00204 L/kg
Kidney	0.00300 L/kg	Plasma	0.02553 L/kg
Liver	0.03011 L/kg	Adipose	0.00274 L/kg

Human WT : 70kg Vss : 0.59 L/kg

4. Hepatic CL in the Elimination tab

- Basic assumption is that the drug is eliminated via hepatic metabolism or renal excretion.
- Hepatic CL is calculated using the well-stirred model ($CL_{hepatic} = \frac{Q \times f_u \cdot CL_{int}}{Q + f_u \cdot CL_{int}}$).
- With the Q and fu already known, obtaining credible CL_{int} value is critical in predicting CL_h (hepatic CL).
- Two experimental methods to measure CL_{int}: the user should opt for either of the two methods: microsome or hepatocyte

1) When using human microsome

- When using microsome, the CL_{int_ini} input by users may be used to calculate hepatic CL (CL_h), or further corrected.

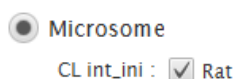
- The microsome protein concentration should also be input.
 - Three kinds of CL (CL_h, CL_{fromRativivc}, and CL_{PS}) are automatically calculated from the CL_{int_ini}. The user can select one of the three human CL values for human PK prediction. It is a decision of the user.
- (1) **CL_h**: In the microsome experiment, when the user inputs microsome intrinsic CL (CL_{int_ini}), it is then corrected for nonspecific binding fraction predicted by 'logD' or 'logP' value [6] or user-input. Then, the fu corrected value is scaled up by the amount of protein per gram liver and weight of liver in a 70 kg human to predict the human hepatic CL (CL_h).
 - (2) **CL_{PS}**: As intrinsic CL can be over-predicted in microsome experiments, passive diffusion CL is applied to intrinsic metabolic CL (CL_{int_ini}) to reflect the diffusion rate of drug molecules across the hepatocyte cell membrane.

- Passive diffusion CL is calculated using the apparent passive permeability

(Papp) of MDCK-II cell (the information input in the Absorption tabpane) and surface area of human hepatocytes. [7].

- When the Papp of MDCK-II is not available, Papp of Caco-2 is used instead. A linear correlation exists between the two Papps, and that of Caco-2 is converted to that of MDCK-II using a linear equation. The equation was developed by PIPET researchers after extensive reference searches [Unpublished data].
- The CL_PS is also calculated from logD (the information input in the Basic info tabpane) without using Papps: correlation between logD and Papp of MDCK-II is used. [7] Then, the CLh_PS is denoted as "CLhPS(logD)" in the final parameters tabpane.

- (3) **CL_fromRativivc:** The CLh is also corrected using rat in vitro-in vivo correlation (ivivc) data with the "rat" checked as below.).



When both of rat microsomal metabolism data (CLint_ini of the rat) and rat *in vivo* hepatic CL (CLh_ratinvivo) are available, their ratio may be applied to obtain corrected human hepatic CL. [8]

- A. First, corrected CLint_ini of rat (CLint_corrected) is back-calculated from observed CLh of rat (CLh_ratinvivo) using the well-stirred model.

$$CL_{-ratinvivo} = \frac{Q \times f_u \cdot CL_{int_corrected}}{Q + f_u \cdot CL_{int_corrected}} \Rightarrow CL_{int_corrected} = ?$$

- B. Second, the ratio between the CLint_ini and CLint_corrected in the rat is calculated.
- C. Third, the ratio is used to calculate the human's CLint_corrected.
- D. Last, the human CLint_corrected is used to calculate CLh_fromRativivc using the well-stirred model equation.
- (4) Correction using rat ivivc is not provided for CLh_PS or CLh_PS(logD) because experiments on rat hepatocyte permeability are not generally done.

2) when using human hepatocyte data

☒ Hepatocyte

CL_{int_ini} : Human ul/min/10⁶ cell

V_{cell} / V_{incubation}

fu_{hep}

CL_h L/hr

- (1) The hepatocyte cell volume ratio can be input considering user's experimental condition. Generally, it is considered to be 0.005 when 10⁶ hepatocyte cells were used.
- (2) When the user inputs intrinsic CL of hepatocyte, it is corrected by nonspecific binding fraction predicted by 'logD' or 'logP' value [6] and scaled up using the number of hepatocytes per gram liver and weight of liver to predict the intrinsic metabolic CL.
- (3) The CL_{h_fromRativivc} is not provided because in vitro metabolism study using rat hepatocytes is rarely performed.
- (4) The CL_{h_PS} is not provided because passing through the hepatocyte cell membrane was already reflected to the CL_{int_ini} obtained from the human hepatocyte experiment.

5. Hepatic CL in the Elimination tab

- 1) Renal CL of human is predicted directly from the rat or dog renal CL corrected by fraction unbound in plasma and kidney blood flow of each animal. [9]
- 2) If the user wishes to apply assumed human renal CL value of the new molecule from information on the same category drugs or else, the user may directly input the value in the Final parameters tabpane.
- 3) The user can select one of the renal CL predicted from animals or directly input by the user (assumed human renal CL) for calculating total CL in the human in the Final parameters tabpane.

6. Final Parameters tab

- 1) The user may choose preferred hepatic CL, Fh, renal CL and Fa values for simulation among several differently calculated parameters in the previous tabpanes.
- 2) Since Fh value is derived from hepatic CL, Fh and hepatic CL are paired, and the Fh value will be automatically selected when one of the hepatic CL values is selected.
- 3) The user has four pairs of choices in hepatic CL and Fh (CLh, CLh_fromRativivc, CL_PS, CL_PS(logD) and corresponding Fh values) when using microsome intrinsic CL.

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Basic Info Absorption Distribution Elimination **Final Parameters** Concentration Prediction About

CL : 72.39 L/hr

Hepatic CL(Microsome)

- ☒ CLh 67.5 L/hr
- ☐ CLh_fromRativivc NaN L/hr
- ☐ CLh_PS 1.14 L/hr
- ☐ CLh_PS(logD) 0.81 L/hr

Renal CL

- ☒ CLr (Rat to human prediction) 4.84 L/hr
- ☐ CLr (Dog to human prediction) 9.16 L/hr
- ☐ CLr (User input) 0.0 L/hr

F : 0.21356

Fh(Microsome)

- ☒ Fh(CLh) 0.22
- ☐ Fh(CLh_fromRativivc) NaN
- ☐ Fh(CLh_PS) 0.98
- ☐ Fh(CLh_PS(logD)) 0.99

Fa

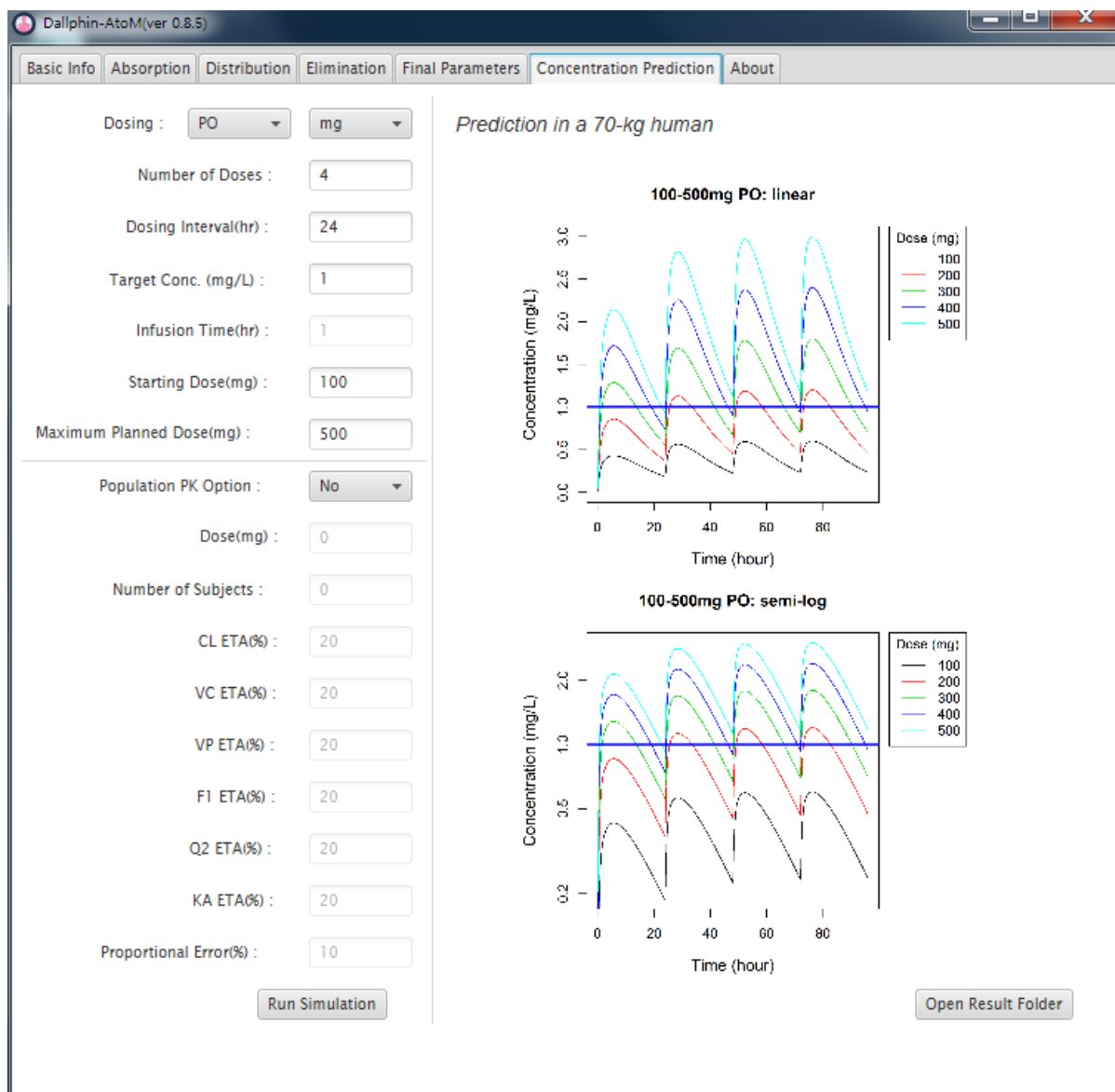
- ☒ Fa_Caco 0.95
- ☐ Fa_PSA 1.0

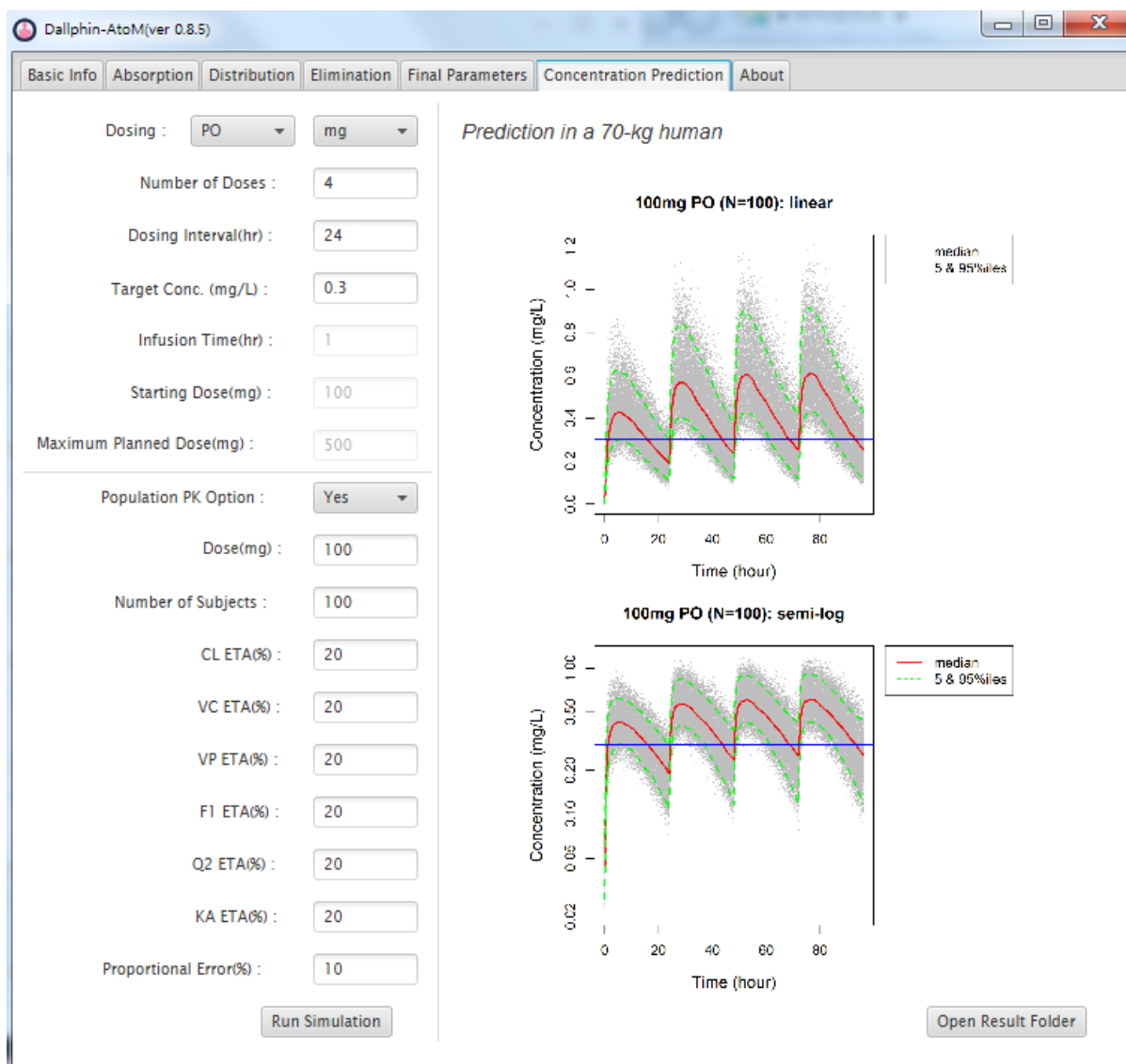
- 4) When using hepatocyte intrinsic CL, there is no choice but one pair of CLh an Fh.

- 5) The user may select one of the two renal CL (from rats or dogs) or enter the assumed renal CL value for simulation.

7. Concentration Prediction tab

- 1) This is the location where the goal of all the input data and parameters is finally harvested.
- 2) Human plasma concentration-time curves after oral or i.v. dosing are simulated using the dose, interval, number of doses input by the user and the final human PK parameters. If necessary, population PK distribution may also be simulated using assumed between-subject variability and residual error values.
- 3) Results and input parameters are also saved in the result folder ('Open Result Folder').





References

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