

Introduction to Dallphin-AtoM (ver. 0.8.8)

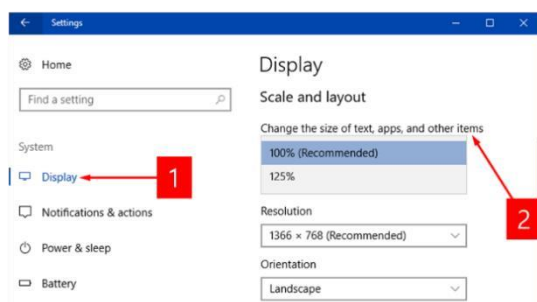
If you are using a laptop or an internal or external monitor with Ultra High Definition (4k UHD) using a higher resolution, you might experience the following problems in Dallphin.

- 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.

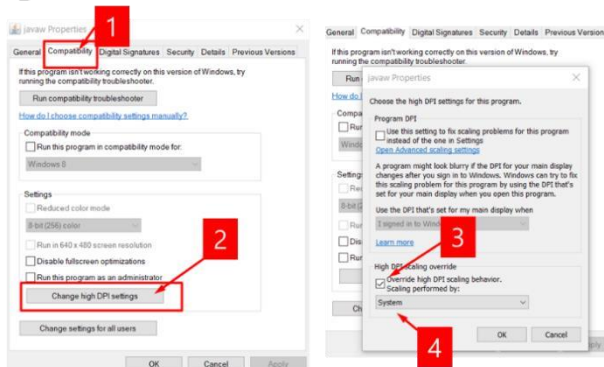
You can solve these problems by applying one of the following solutions.

1. Change the size of apps: You can rightclick on the Desktop (background image) > Display Settings > 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-Phin\Wire\bin in the Explorer and follow these actions: Right click > Properties > Compatibility tab > Change high dpi settings > Click override system settings. (Figure 1B)

A



B



1. What is Dallphin-AtoM?

- Dallphin is the acronym of the phrase 'Drugs with **ALL**ometry and **PH**ysiology **INS**ide' and AtoM is 'Animal to human.'



- 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) 0.8.8 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 0.8.8 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 0.8.8 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.

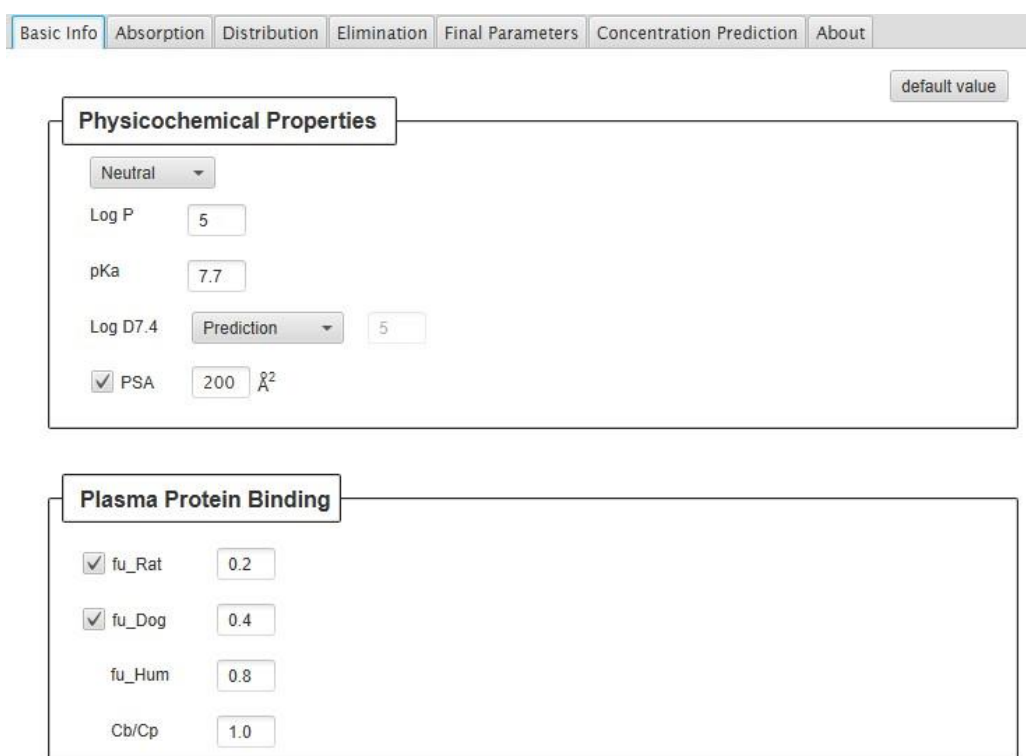
6. What this manual tells

- 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 0.8.8)



- When Dallphin is launched in your computer, you will 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.



The screenshot shows the 'Basic Info' tab of the Dallphin software. The top navigation bar includes tabs for 'Basic Info', 'Absorption', 'Distribution', 'Elimination', 'Final Parameters', 'Concentration Prediction', and 'About'. The 'Basic Info' tab is active, displaying two main sections: 'Physicochemical Properties' and 'Plasma Protein Binding'. In the 'Physicochemical Properties' section, there is a dropdown menu set to 'Neutral', input fields for 'Log P' (5), 'pKa' (7.7), 'Log D7.4' (Prediction, 5), and a checked checkbox for 'PSA' (200 Å²). A 'default value' button is located at the top right of this section. The 'Plasma Protein Binding' section contains input fields for 'fu_Rat' (0.2), 'fu_Dog' (0.4), 'fu_Hum' (0.8), and 'Cb/Cp' (1.0), each with a corresponding checked checkbox.

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]
- 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 Fa calculated herein is given as Fa_Caco in the bottom of the pane.

2) Fg prediction

- Fg (intestinal availability) was predicted using the Qgut model as defined in the following equation when the drug is metabolized by CYP3A. [4]

$$Q_{gut} = \frac{CL_{perm} * Q_{ent}}{CL_{perm} + Q_{ent}}$$

- Q_{gut} is a hybrid parameter of blood flow and drug permeability and calculated using Caco-2 apparent permeability, human intestinal surface area, and mucosal blood flow.
- F_g is calculated using Q_{gut} , unbound fraction in the gut, and intrinsic clearance in intestinal microsome. For convenience, unbound fraction in the gut is fixed to 1.
- Intrinsic clearance in intestinal microsome can be directly input using in vitro experiment data or predicted from intrinsic clearance for the CYP3A pathway in liver microsome by scaling the abundance of CYP3A in liver and intestine.
- F_g is considered to be 1 when the given drug is not metabolized by CYP3A or intestinal clearance

3) MDCK permeability

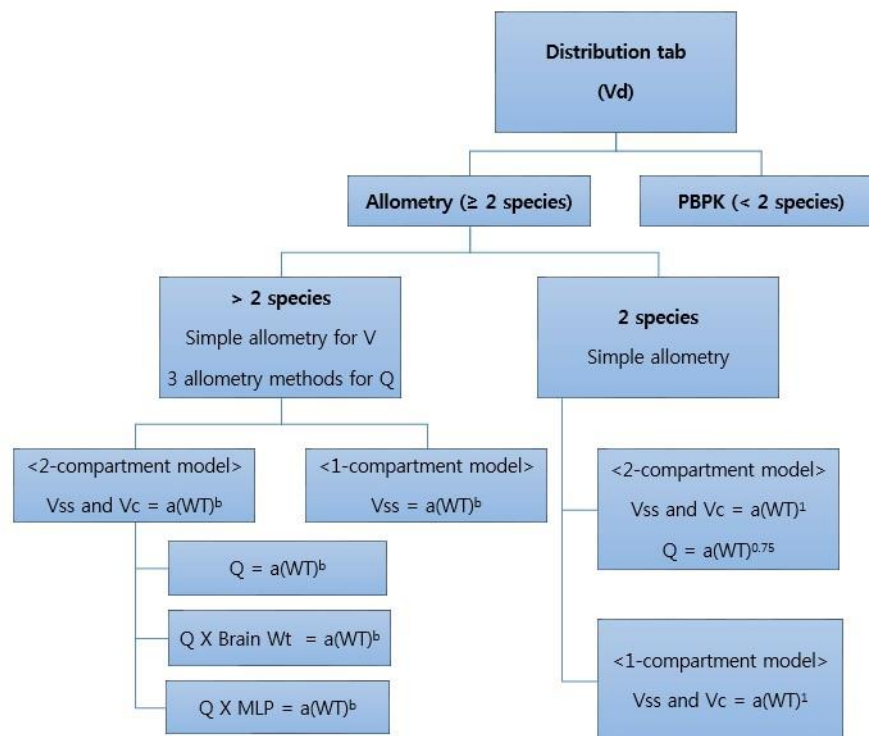
- MDCK-II cell permeability may be input here when available. It is used for the calculation of microsome-data based hepatic CL (Elimination tabpane).

4) 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.

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 of the two methods.



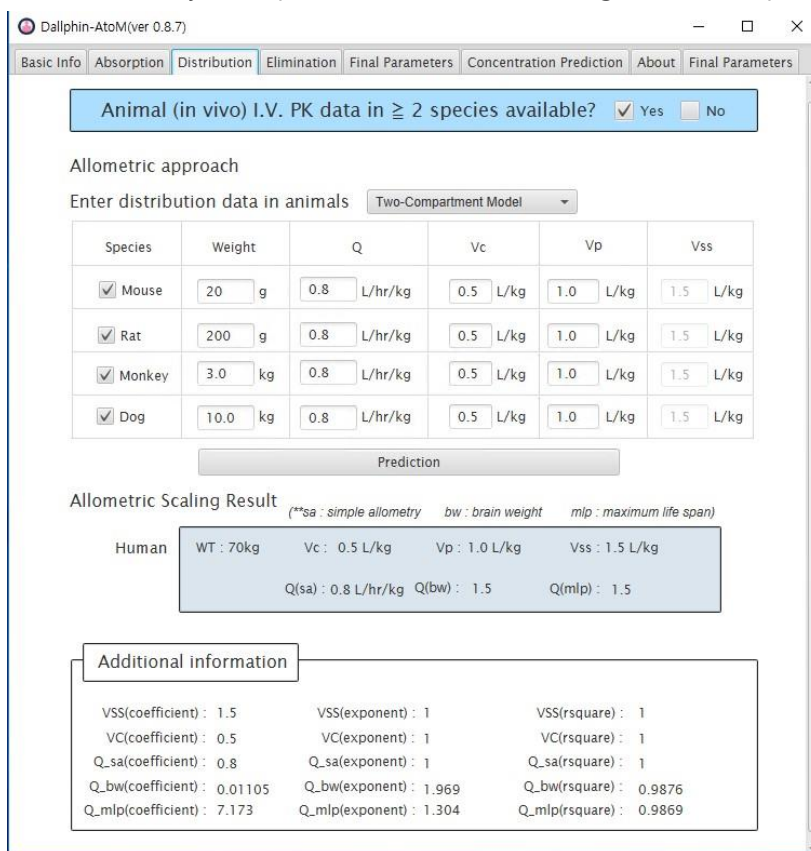
1) when using the allometric methods

- 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 necessary to use the allometric methods.
- V_c , V_p , V_{ss} in a 70-kg human are estimated using simple allometry, and coefficients (a), exponents (b) and R^2 values of the best-fit lines ($V = a(WT)^b$, R^2 = coefficient of determination) are reported. [5,6]
- Q (intercompartmental CL) is estimated in three allometric methods, and coefficients (a), exponents (b) and R^2 values of the best-fit lines are reported so that the user may select one of the following: [5-7]
 - Simple allometry ($Q = a(WT)^b$)
 - Allometry with correction factor using brain weight ($Q \times \text{Brain Weight} = a(WT)^b$)
 - Allometry with correction factor using MLP (product of maximum life-span) ($Q \times \text{MLP} = a(WT)^b$)

- *Dallphin uses default brain weight and MLP for each species. [8] However, when i.v. PK data available in only two species, Dallphin estimates Vc, Vp, Vss, and Q of a 70-kg human using simple allometry with a fixed exponent of 1 for volume parameters and 0.75 for Q.
- As for the compartmental model selection, the user may choose either one- or two-compartment, 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 the 2-compartment model:

- Vc and Vp in each species are to be input by users and Vss is automatically filled up ($V_{ss} = V_p + V_c$).
- Dallphin calculates human Vss and Vc allometrically, and reports estimated coefficients, exponents, and R^2 values when i.v. PK data available in ≥ 3 species, however when data is available in only two species, it is calculated using the fixed exponent of 1 to body weight



Dallphin-AtoM(ver 0.8.7)

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

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	Vc	Vp	Vss
<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 (**sa : simple allometry bw : brain weight mlp : maximum life span)

Human WT : 70kg Vc : 0.5 L/kg Vp : 1.0 L/kg Vss : 1.5 L/kg
Q(sa) : 0.8 L/hr/kg Q(bw) : 1.5 Q(mlp) : 1.5

Additional information

VSS(coefficient) : 1.5	VSS(exponent) : 1	VSS(rsquare) : 1
VC(coefficient) : 0.5	VC(exponent) : 1	VC(rsquare) : 1
Q_sa(coefficient) : 0.8	Q_sa(exponent) : 1	Q_sa(rsquare) : 1
Q_bw(coefficient) : 0.01105	Q_bw(exponent) : 1.969	Q_bw(rsquare) : 0.9876
Q_mlp(coefficient) : 7.173	Q_mlp(exponent) : 1.304	Q_mlp(rsquare) : 0.9869

(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 is strongly recommended!). In this case, V_{ss} is the only parameter to be entered and estimated in a 70-kg human (Neither V_p nor Q are required).
- The method to calculate human V_{ss} is the same as above for volume parameter calculations using simple allometry.
- Occasionally, the i.v. PK data may be better fitted by a 1-compartment model according to the PK sampling scheme, infusion time, etc. 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 V_{ss} obtained from the non-compartmental analysis by selecting the "1-compartment" model.
- .

2) when using the PBPK method

- If the animal in vivo PK data is not available, V_{ss} 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. [9-11]
- All of the physiological variables from the references are used in Dallphin AtoM as built-in equations to calculate V_{ss} .
- When using the PBPK method, only V_{ss} is given to the user (without presenting V_c and V_p separately) as if the drug follows a 1-compartment model.
- Although the V_c 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 V_c is obtained.)

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

PBPK approach

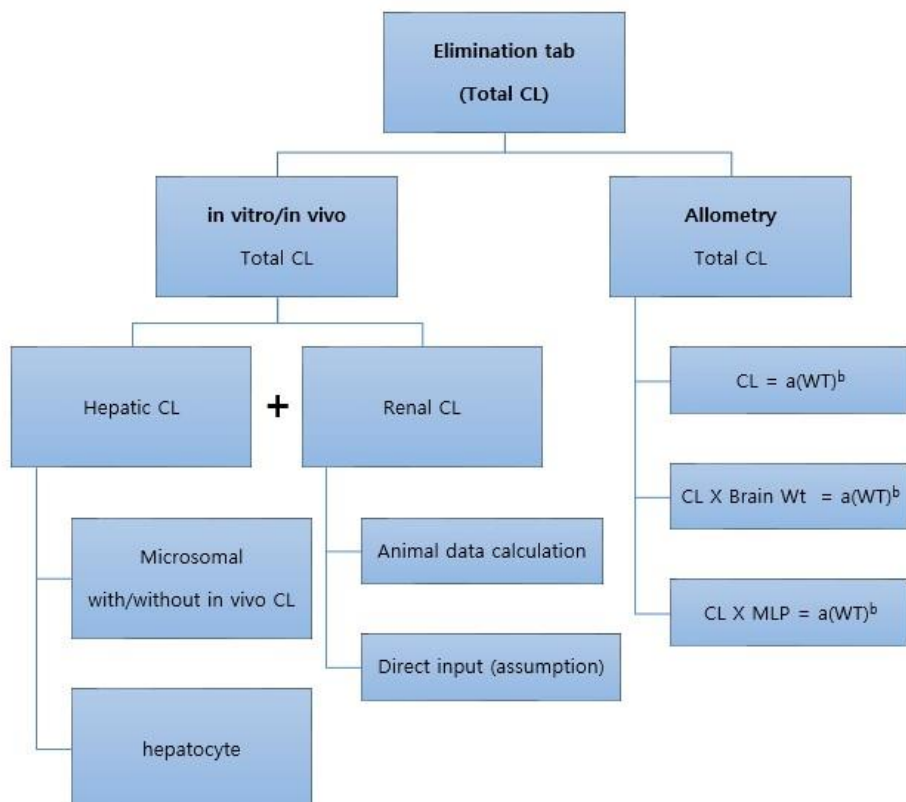
Predicted human tissue distribution One-Compartment Model

Bone	1.8884 L/kg	Lung	0.0194 L/kg
Brain	0.3767 L/kg	Muscle	3.0453 L/kg
Cut	0.3683 L/kg	Skin	0.6641 L/kg
Heart	0.0186 L/kg	Spleen	0.0196 L/kg
Kidney	0.0292 L/kg	Plasma	0.0445 L/kg
Liver	0.3976 L/kg	Adipose	29.143 L/kg
Thymus	0.0026 L/kg		

Human WT : 70kg Vss : 36.02 L/kg

4. Elimination tab – choices in Elimination tab

You may opt for either of in vitro/in vivo or allometric approach to predict human CL.



5. 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 ($CL_{hepatic} = \frac{Q \times f_u \times CL_{int}}{Q + f_u \times CL_{int}}$).
- 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

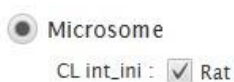
- When using microsome, the CL_{int_ini} input by users may be used to calculate hepatic CL (CL_h), or further corrected.

- 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 userinput. 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. [12]
- 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 MDCKII is used. [12] 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. [13]

- 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, human CLint_corrected is used to calculate CLh_fromRativivc using the well-stirred model equation.

- (4) Correction using rat ivivc is not provided for CL_h_PS or CL_h_PS(logD) because experiments on rat hepatocyte permeability are not generally done.

2) when using human hepatocyte data



☒ Hepatocyte

CL int_ini : Human 115 ul/min/10⁶ cell

V_{cell} / V_{incubation} 0.005

fu_{hep} Prediction 0.75

CL_h 70.6 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 [10] 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. Renal 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. [14]
- 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. Total CL in the Elimination tab using the allometry methods

- 1) The user may select the allometric approach to calculate CL in Elimination tab and input CL for each species acquired from compartmental models from either 1- or 2-compartment model.
- 2) Dallphin calculates human CL in three allometric methods and reports coefficients (a), exponents (b) and R^2 values of the best-fit lines, which enables the user to select results from one of the following: [5-7]
 - Simple allometry ($CL = a(WT)^b$)
 - Allometry with correction factor using brain weight ($CL \times \text{Brain Weight} = a(WT)^b$)
 - Allometry with correction factor using MLP (product of maximum life-span) ($CL \times \text{MLP} = a(WT)^b$)
- 3) However, when i.v. PK data available in only two species, Dallphin estimates CL of a 70-kg human using simple allometry with a fixed exponent of 0.75.
- 4) When one of three allometric methods is chosen for human CL, the same method is automatically chosen for human Q.

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

☐ in vitro / in vivo ☒ allometric approach

Allometric approach
Enter clearance data in animals

Species	Weight	CL
<input checked="" type="checkbox"/> Mouse	20 g	2.5 L/hr
<input checked="" type="checkbox"/> Rat	200 g	11.0 L/hr
<input checked="" type="checkbox"/> Monkey	3 kg	1.0 L/hr
<input checked="" type="checkbox"/> Dog	5 kg	1.0 L/hr

Prediction

Allometric scaling result (**sa : simple allometry bw : brain weight mlp : maximum life span)

WT : 70kg CL(sa) : 1.501 CL(bw) : 0.000001719CL(mlp) : 0.571

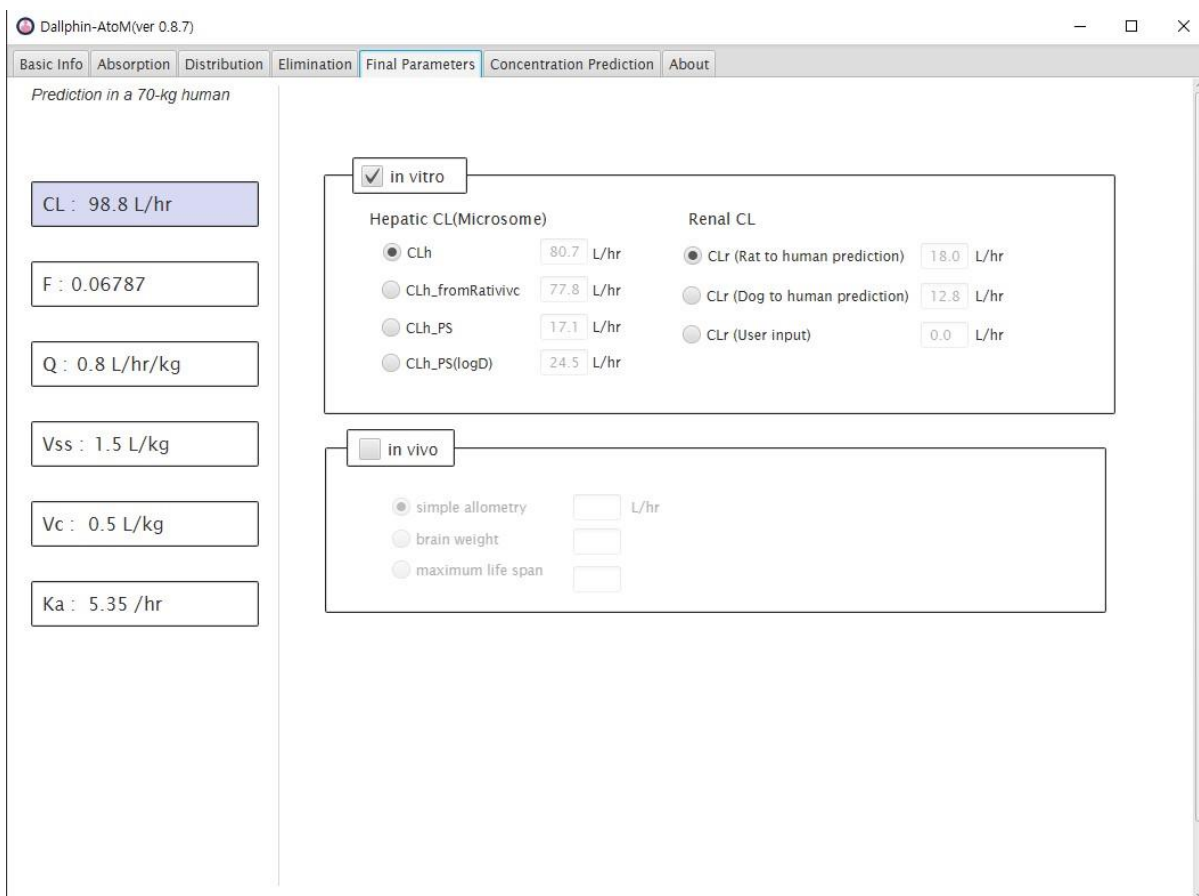
Additional information

CL_sa(coefficient) : 1.373	CL_sa(exponent) : 1.021	CL_sa(rsquare) : 0.9464
CL_bw(coefficient) : 0.00...	CL_bw(exponent) : -0.0...	CL_bw(rsquare) : 0.00...
CL_mlp(coefficient) : 2.377	CL_mlp(exponent) : 0.6643	CL_mlp(rsquare) : 0.9586

7. Final Parameters tab

- 1) The user may choose preferred total CL (1. in vitro/in vivo approach (hepatic CL + renal CL), or 2. Allometric approach), Fh, renal CL, and Fa values for simulation among several differently calculated parameters in the previous tabpanes.
- 2) Since the Fh is derived from hepatic CL, Fh and hepatic CL are paired, and the Fh will be automatically selected when one of the hepatic CL values is selected.
- 3) For in vitro/in vivo approach CL calculation, the user has four pairs of choices in hepatic CL and Fh (CLh, CL_fromRativivc, CL_PS, CL_PS(logD) and corresponding Fh values) when using microsome intrinsic CL.
- 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 the assumed renal CL value for simulation.
- 6) For allometry approach CL calculation, the user has three choices to select.
- 7) By clicking PK parameters on the left, all the methods/selections are available so the user

can choose the final calculation methods for each PK parameters.



Dallphin-AtoM(ver 0.8.7)

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

Prediction in a 70-kg human

CL : 98.8 L/hr

F : 0.06787

Q : 0.8 L/hr/kg

Vss : 1.5 L/kg

Vc : 0.5 L/kg

Ka : 5.35 /hr

☒ in vitro

Hepatic CL(Microsome)

- ☒ CLh 80.7 L/hr
- ☐ CLh_fromRativivc 77.8 L/hr
- ☐ CLh_PS 17.1 L/hr
- ☐ CLh_PS(logD) 24.5 L/hr

Renal CL

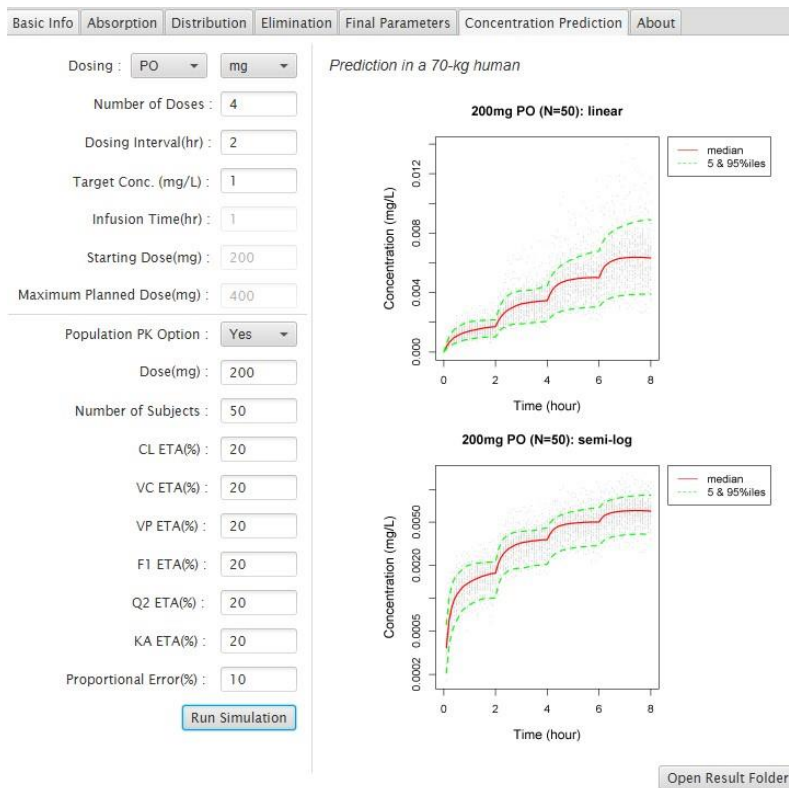
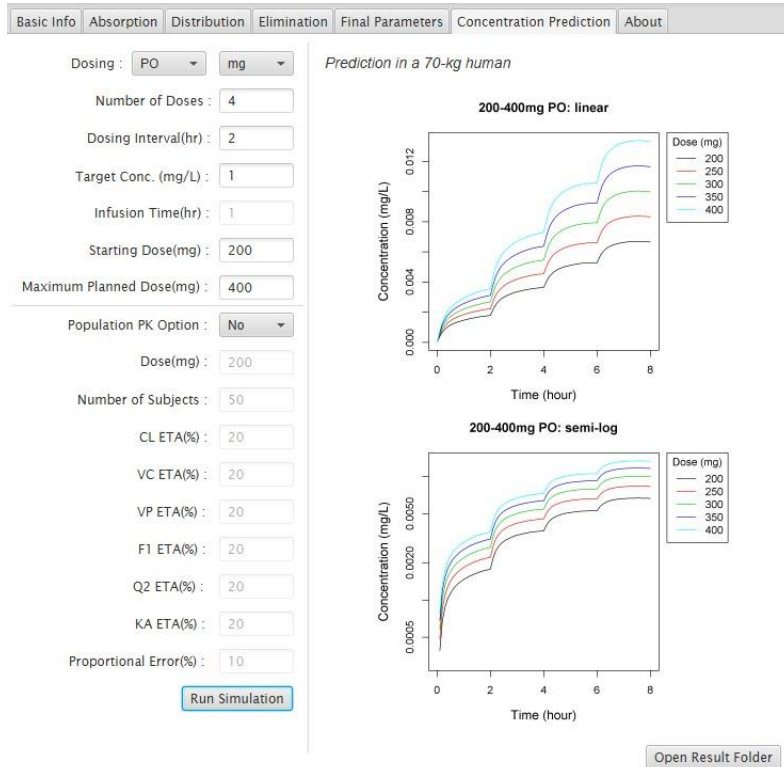
- ☒ CLr (Rat to human prediction) 18.0 L/hr
- ☐ CLr (Dog to human prediction) 12.8 L/hr
- ☐ CLr (User input) 0.0 L/hr

☐ in vivo

- ☒ simple allometry [] L/hr
- ☐ brain weight []
- ☐ maximum life span []

8. 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').



Release note (0.8.8)

1. Elimination tab: The error in the body weight unit at the allometric CL prediction step was fixed.
2. Final Parameter tab: The error in the display of Vss for a 1-compartment model was fixed.
3. Distribution tab: The error in using the brain weight and MLP at the allometric Q prediction step was fixed.
4. Distribution tab: At the allometric 1-compartment model, the k_a and Fa_{Caco} are automatically given like in the case of the 2-compartment model.
5. JRE (Java Runtime Environment) was changed from the Oracle JRE to the open JRE.
6. Prediction button set disable when prediction is performing for prevent request repetition.

Release note (0.8.7)

1. The Qgut model was included to predict the intestinal bioavailability (F_g)
2. Allometric calculation methods of CL was added.

Release note (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.



References

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