Introduction to DallphinAtoM (ver. 0.9.4)

1. What is DallphinAtoM?

- DallphinAtoM is the acronym of the phrase '**D**rugs with **ALL**ometry and **PH**ysiology **In**side - **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.
- The current beta version is for Windows (x64).

2. Why DallphinAtoM?

- There are several commercial and free PBPK software packages already available for human PK prediction. However, we felt needs for a software package that does not request overly many input data and that clarifies the calculation methods or references to users as well as being freely available. Thus, DallphinAtoM was born.
- Detailed theories and *in vitro*/PBPK methods applied to develop DallphinAtoM are reviewed in three tutorials published by PIPET (Pharmacometrics Institute for Practical Education and Training). [1-3]

3. Right to use DallphinAtoM

- DallphinAtoM (Dallphin, for simplicity) 0.9.4 is distributed to beta testers for test use only.
- Any suggestion, comments or bug reports via email (yimds@catholic.ac.kr) or Github Issues (https://github.com/pipetcpt/dallphin/issues) are welcomed.

4. Who developed DallphinAtoM?

- DallphinAtoM 0.9.4 was developed by researchers of PIPET of the College of Medicine, the Catholic University of Korea for the education of students and researchers.
- DallphinAtoM 0.9.4 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 DallphinAtoM 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 DallphinAtoM is dependent on the references that were used in DallphinAtoM. Under no circumstances shall the developers of DallphinAtoM have any liability to the users for any loss or damage of any kind incurred as a result of the use of DallphinAtoM or reliance on any information provided by DallphinAtoM.
- The user may not modify contents of the Dallphin, nor distribute the modified installation files.

6. What this manual tells

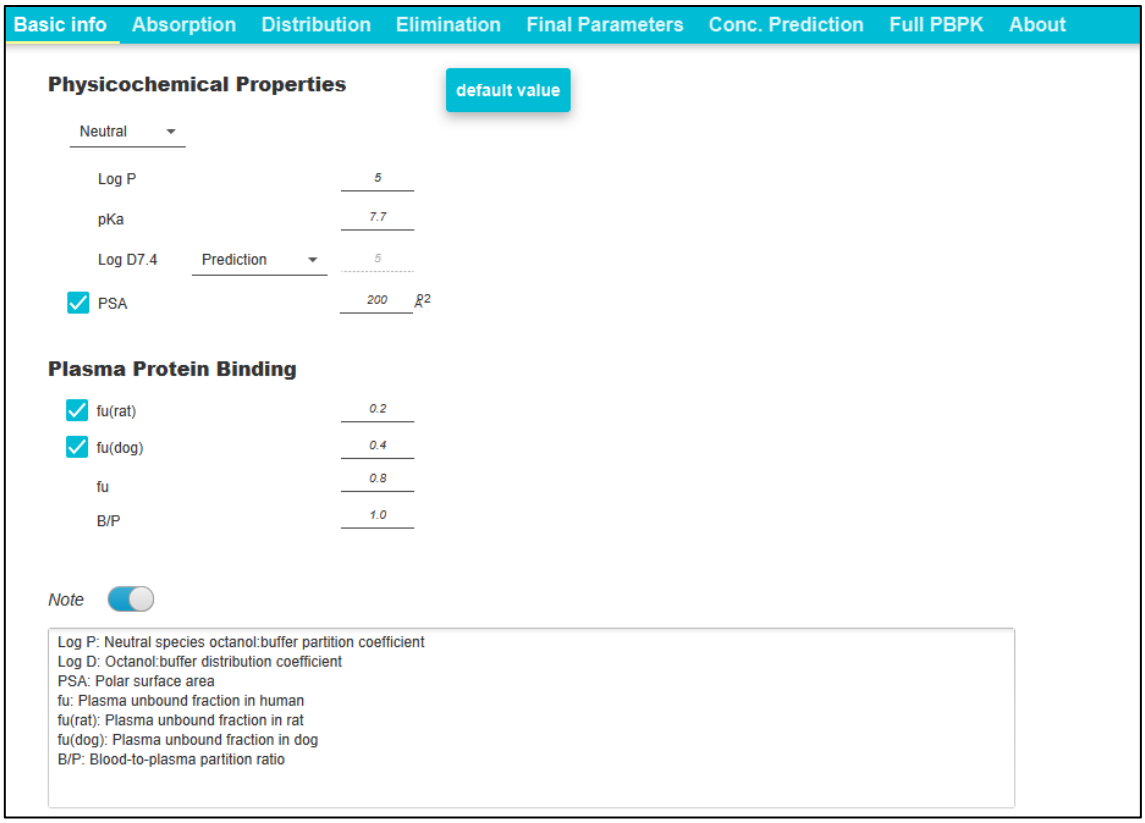
- To predict drug exposure in human, we need to know four major PK parameters - k_a, V_d (V_c, V_p, and Q), CL and F if the drug follows first-order kinetics.
- This manual briefly introduces the principles used in the prediction of each of the four PK parameters based on in vitro or animal PK data together with corresponding references.

Brief manual for DallphinAtoM (ver 0.9.4)

- When DallphinAtoM is launched in your computer, you will see eight 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 (Conc.) Prediction" or the predicted plasma and tissue concentrations at the "Full PBPK" tabpane.



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.

- LogD may be directly inputted by the user or calculated from logP and pKa values.

- The physicochemical property inputs are used at the calculation of the 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, F_a calculated using PSA [4] is shown at the Absorption tabpane as Fa_PSA.

2. Absorption Tab

- The *in vitro* and PBPK methods applied to predict oral drug absorption in DallphinAtoM were reviewed in the tutorial published by PIPET. [1]

1) Caco-2 permeability and Fa

- Caco-2 permeability of the test drug should be input as P_{app}. Those from reference standard drugs (propranolol and/or atenolol) are also recommended to input to calibrate the test drug's P_{app} in a bid to minimize the inter-laboratory or inter-occasional differences in P_{app} assay data.
    - In DallphinAtoM, the calibration method is based on the F_a predicted using the equation proposed by Usansky et al. [5] The reference P_{app} values of propranolol (21.4×10^{-6} cm/sec) and atenolol (0.37×10^{-6} cm/sec) are those expected to give the predicted F_a identical to known F_a values of propranolol (90%) and atenolol (56%), respectively. The ratios of user-input P_{app} versus the ideal P_{app} of the two reference drugs are used to calibrate the P_{app} of the test drug.

- The P_{eff} (permeability in human duodenum) is calculated from the P_{app} (or corrected P_{app} if P_{app} of reference drugs are available) which is in vitro data. [6] The F_a is then calculated using the estimated P_{eff} and the ratio of the transit time and the radius of human small intestine. [7] The calculated F_a herein is given as Fa(Caco) in the "Final Parameters" tab.

- Also, the F_a is calculated using equations on the relationship between the estimated first-order absorption rate constant (Refer to section 2.4)) and the rate constant of intestinal transit. [5] The F_a calculated herein is given as Fa(Caco, ki) in the "Final Parameters" tab.

- Thus, once the Caco-2 permeability value of the test drug is provided, both Fa(Caco) and Fa(Caco, ki) values are shown in "Final parameters" tab, and the user may choose either of the two F_a's for the simulation.

2) F_g prediction

- F_g (intestinal availability) was predicted using the Q_{gut} model as defined in the following equation when the drug is metabolized by CYP3A. [8]

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

- 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 the liver and intestine.

- F_g is considered to be 1 when the instrinsic clearance by liver/intestinal microsomal CYP3A is not provided.

3) MDCK permeability

- MDCK-II cell permeability may be input or can be predicted using Caco-2 P_{app}. It is used to estimate passive CL that may be used when the microsomal method is chosen for the calculation of hepatic CL (Elimination tabpane).

4) first-order absorption rate constant: k_a

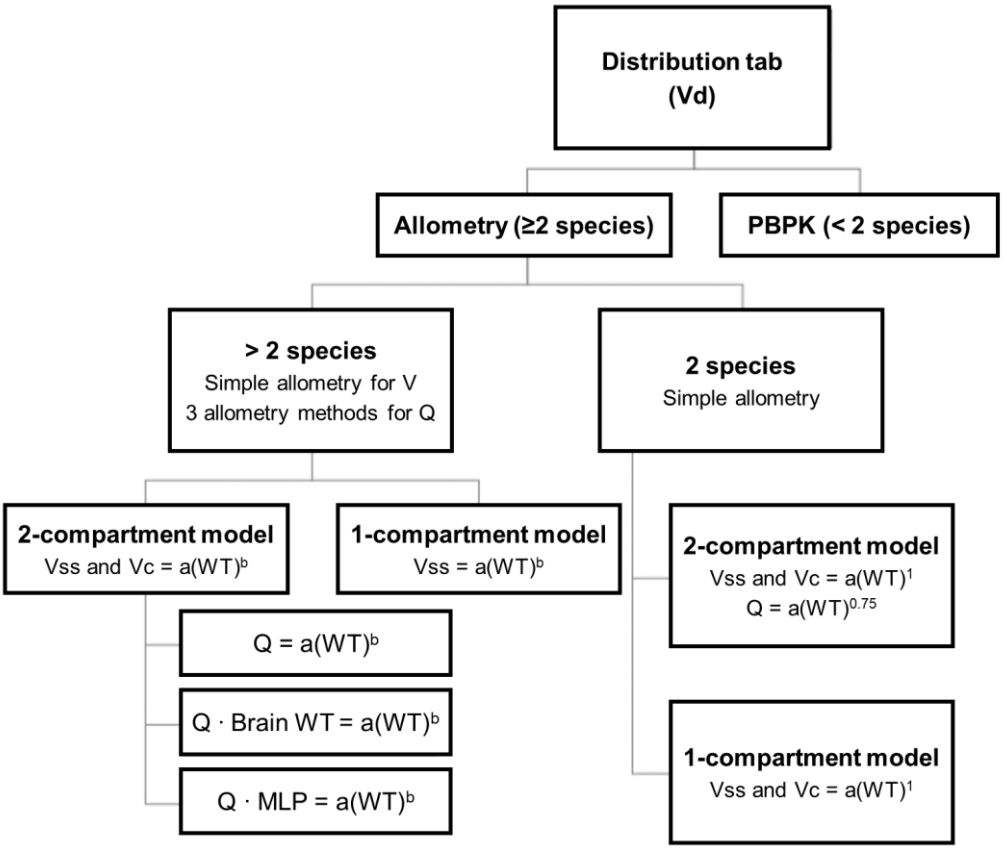
- We used an equation to predict human ka that is calculated from Caco-2 permeability data, intestinal surface area, and V_c (central compartment V_d). [5]

- 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 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 Vd in DallphinAtoM. The user should opt for either of the two methods.

- Background and methods to predict the human volume of distribution (Vd) of drugs using *in vitro* and animal pharmacokinetic (PK) parameters were reviewed in the tutorial published by PIPET. [2]



1) when using the allometric methods

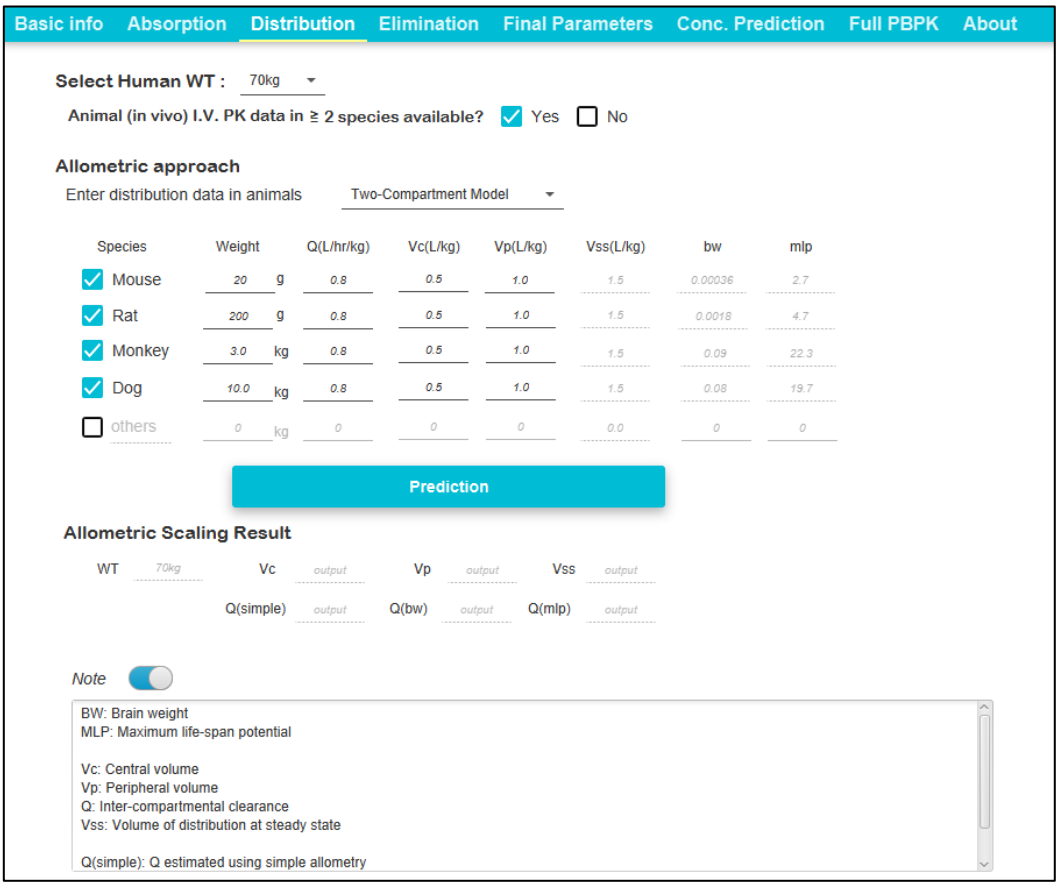
- Compartmental PK parameters on the distribution (V_c, V_p, and Q, the inter-compartmental CL) obtained from intravenous (i.v.) PK study in ≥ 3 animal species (mouse, rat, dog, monkey etc) is necessary to use the allometric methods.

- 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. [9, 10]

- 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: [9-11]
  - 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) ($Q \times \text{MLP} = a(WT)^b$)

- DallphinAtoM uses default brain weight and MLP for each species. [12] However, when i.v. PK data is available in only two species, DallphinAtoM estimates V_c, V_p, V_{ss}, 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:

- V_c and V_p in each species are to be input by users and V_{ss} is automatically filled up ($V_{ss} = V_p + V_c$).

- DallphinAtoM calculates human V_{ss} and V_c 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.

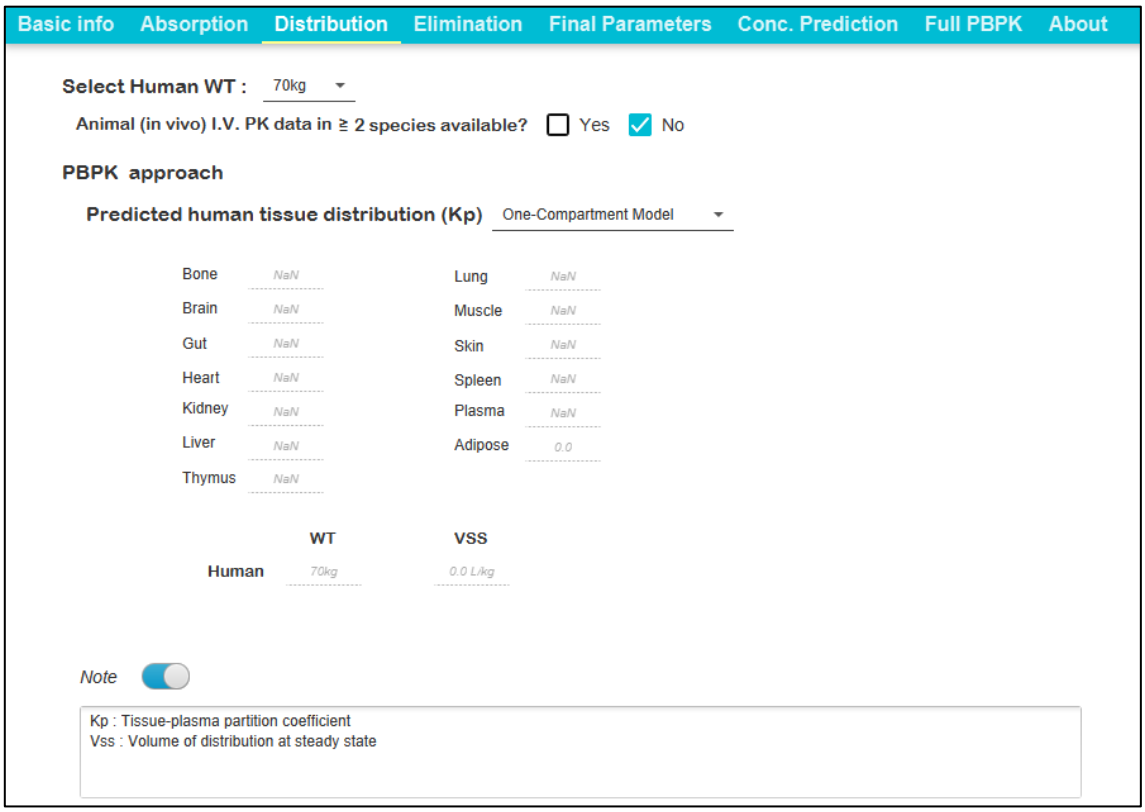
(2) In the case of 1-compartment model:

- 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 for 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. [13-15]

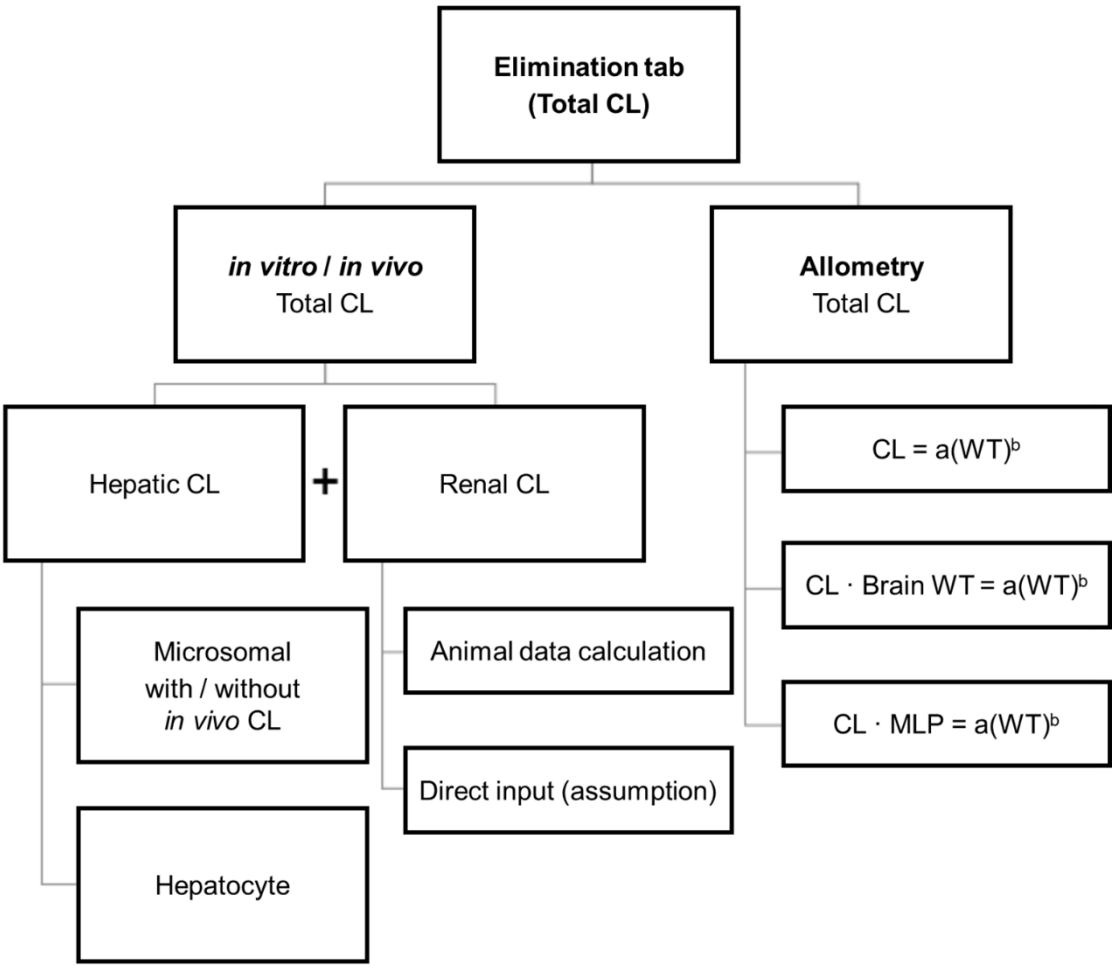
- All of the physiological variables from the references are used in DallphinAtoM as built-in equations to calculate V_{ss}.

- When using the PBPK method, V_{ss} is internally used (without presenting V_c and V_p separately) as if the drug follows a 1-compartment model and predicted human tissue distribution (K_p) values are given to the user.

- 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 despite the calculated V_c)

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

- With the Q and f_u already known, obtaining credible CL_{int} value is critical in predicting CL_h (hepatic CL).

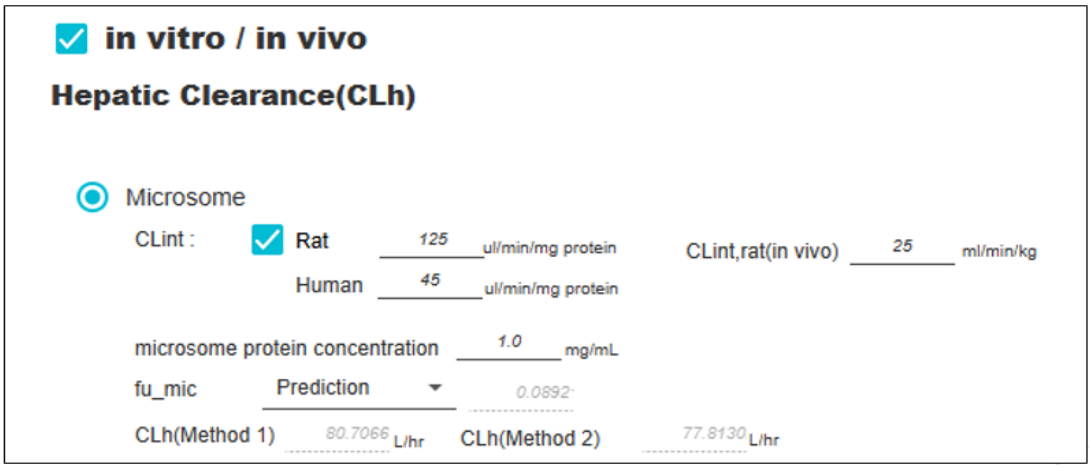
- Two experimental methods to measure CL_{int}: the user should opt for either of the two methods: microsome or hepatocyte.

- When a compound has quite high CL_{int}, its F_h is known to be more accurately predicted by the dispersion model (D_N = 0.17) than by the well-stirred model.[16]

- Various *in vitro* methods applied to predict human CL in DallphinAtoM was reviewed in the tutorial published by PIPET. [3]

1) When using human microsome

- When using microsome, the CL_{int} 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 (Method 1,3,4) are automatically calculated from the CL_{int}. CL (Method 2) is calculated only when the rat in vitro-in vivo correlation data exists. The user can select one of the four human CL values for human PK prediction. It is a decision of the user.

- Method 1: The general IVIVE approach that is obtained using MPPGL and liver weight
- Method 2: The general IVIVE approach (method 1) was calibrated by in vivo clearance observed in rats.
- Method 3: The general IVIVE approach (method 1) was calibrated by passive diffusion clearance estimated using Caco-2 permeability.
- Method 4: The general IVIVE approach (method 1) was calibrated by passive diffusion clearance estimated using logD.

(1) **CLh(Method1)**: In the microsome experiment, when the user inputs microsome intrinsic CL (CLint), 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) **CLh(Method3)**: As intrinsic CL can be over-predicted in microsome experiments, passive diffusion CL is applied to intrinsic metabolic CL (CL_{int}) to reflect the diffusion rate of drug

molecules across the hepatocyte cell membrane.

- Passive diffusion CL is calculated using the apparent passive permeability (P_{app}) of MDCK-II cell (the information input in the Absorption tabpane) and surface area of human hepatocytes. [17]
- When the P_{app} of MDCK-II is not available, P_{app} of Caco-2 is used instead. A linear correlation exists between the two P_{app}, 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].
- Passive diffusion CL is also calculated from logD (the information input in the Basic info tabpane) without using Papps: correlation between logD and P_{app} of MDCK-II is used. [17] Then, the CL_h is denoted as "CLh(Method4)" in the final parameters tabpane.

(3) **CLh(Method2)**: The CL_h 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 (CL_{int} of the rat) and rat *in vivo* hepatic CL ($CL_{h,rat}$(in vivo)) are available, their ratio may be applied to obtain corrected human hepatic CL. [18]

A. First, corrected CL_{int} of rat ($CL_{int,corrected}$) is back-calculated from observed CL_h of rat (CLh,rat(in vivo)) using the well-stirred model.

$$CL_{\_ratinvivo} = \frac{Q \times f_u \cdot CL_{int,corrected}}{Q + f_u \cdot CL_{int,corrected}} \Rightarrow CL_{int,corrected} = ?$$

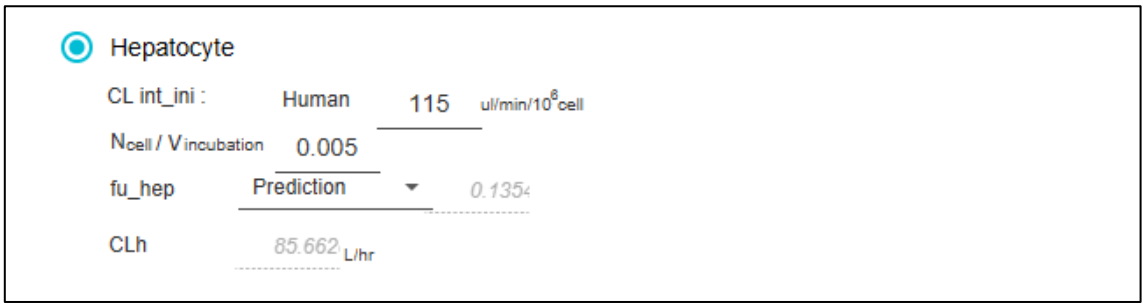
B. Second, the ratio between the CL_{int} and $CL_{int,corrected}$ in the rat is calculated.

C. Third, the ratio is used to calculate the human's $CL_{int,corrected}$.

D. Last, human $CL_{int,corrected}$ is used to calculate CLh(Method 2) using the well-stirred model equation.

(4) Correction using rat ivivc is not provided for CLh(Method 3) or CLh(Method 4) because experiments on rat hepatocyte permeability are not generally done.

2) when using human hepatocyte data



(1) The hepatocyte cell volume ratio can be input considering user's experimental condition. Generally, it is considered to be 0.005 when 10^6 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 [14] and scaled up using the number of hepatocytes per gram liver and weight of liver to predict the CLh(Method1).

(3) The CLh(Method2) is not provided because in vitro metabolism study using rat hepatocytes is rarely performed.

(4) The CLh(Method 3, 4) is not provided because passing through the hepatocyte cell membrane was already reflected to the CL_{int} obtained from the human hepatocyte experiment.

6. 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. [19]

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 user-input value (assumed human renal CL) for calculating total CL in the human in the Final parameters tabpane.

7. Total CL in the Elimination tab using the allometry methods

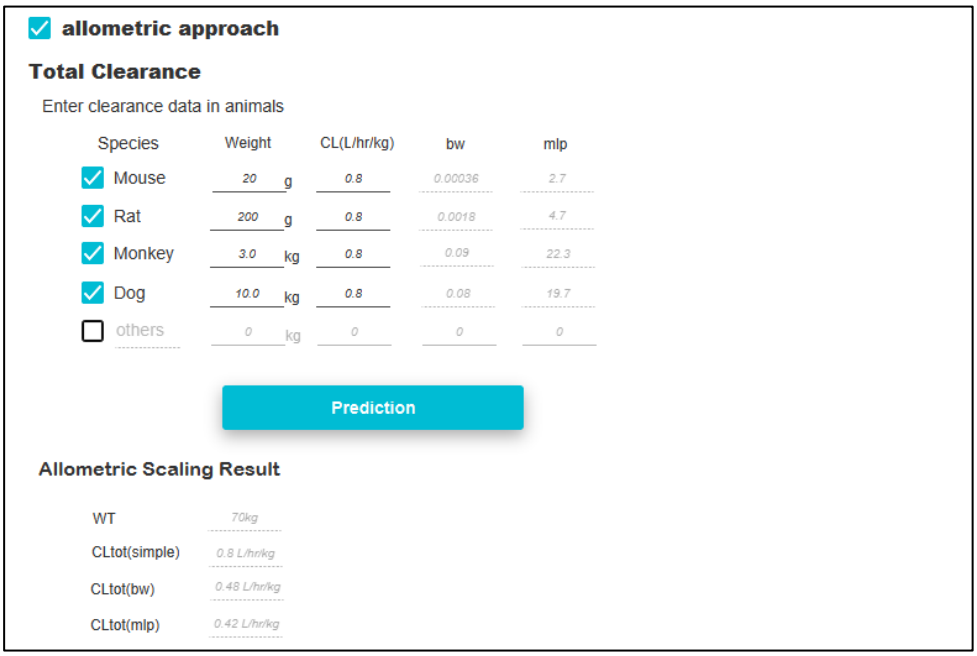
1) The user may select the allometric approach to calculate CL in Elimination tab and input CL for each species acquired from compartmental models from either of the 1- or 2-compartment model.

2) DallphinAtoM 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: [9-11]

   - 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) ($CL \times MLP = a(WT)^b$)

3) However, when i.v. PK data available in only two species, DallphinAtoM 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.



8. Final Parameters tab

1) The user may choose preferred one in the two total CL (1. in vitro/in vivo approach (hepatic CL + renal CL), or 2. Allometric approach), F_h, renal CL, and F_a values for simulation among several differently calculated parameters in the previous tabpanes.

2) Since the F_h is derived from hepatic CL, F_h and hepatic CL are paired, and the F_h will be automatically selected when one of the hepatic CL values is selected.

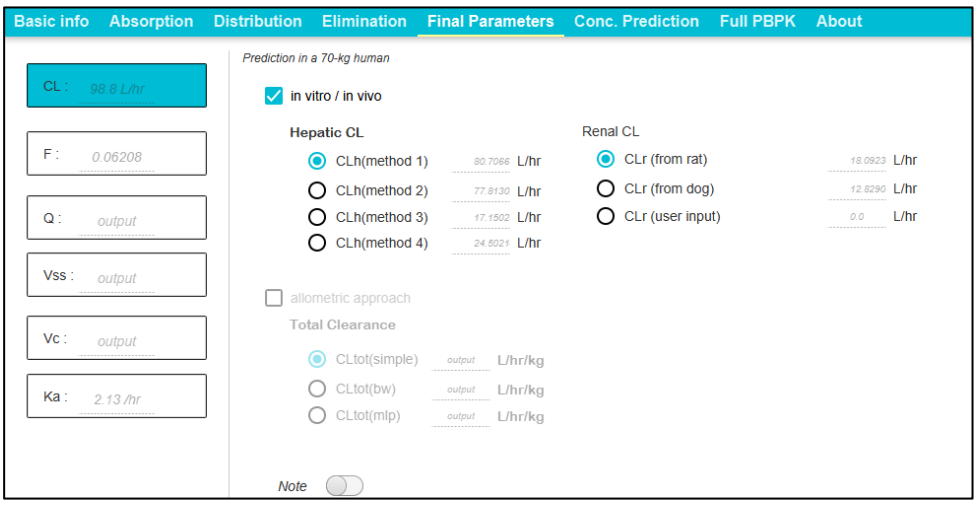
3) For in vitro/in vivo approach CL calculation, the user has four pairs of choices in hepatic CL and F_h (Method 1~4) when using microsome intrinsic CL.

4) When using hepatocyte intrinsic CL, there is no choice but one pair of CL_h an F_h.

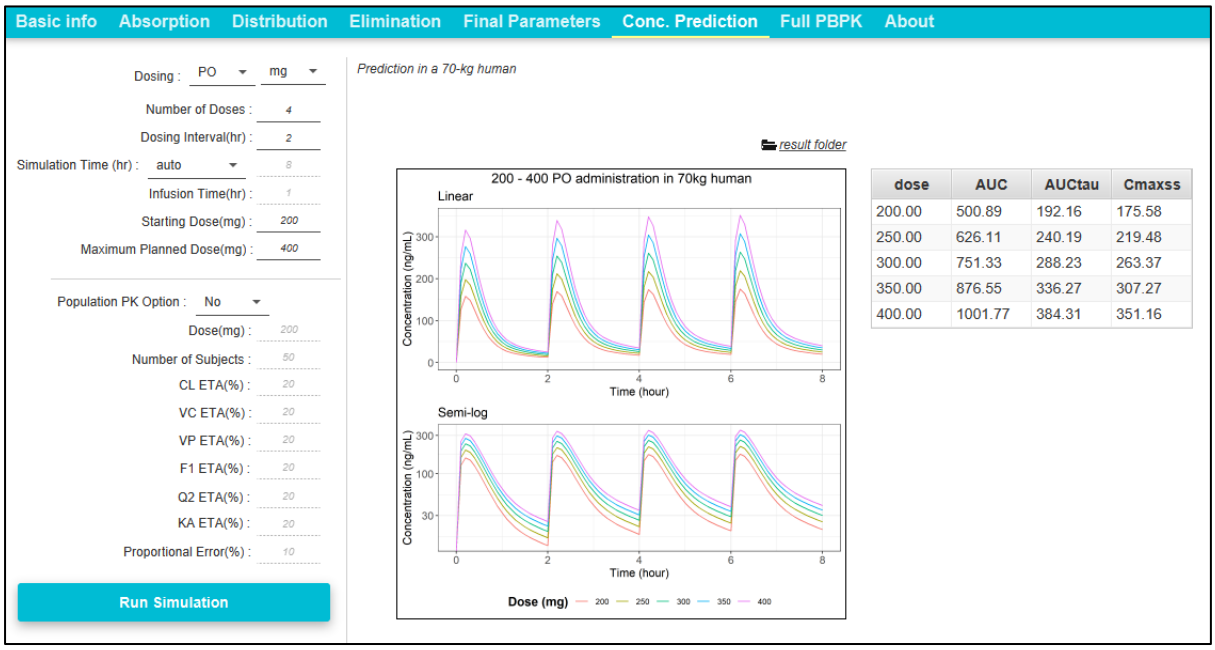
5) The user may select one of the two renal CL (from rats or dogs) or the assumed renal CL value for simulation.

6) For allometry approach CL calculation, the user has three choices.

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.



9. Conc. 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 (simulated datasets), figures and input parameters are also saved in the result folder (Click '*result folder*').

4) The values of the AUC of a single dosing ($AUC_{single\ dose,tau}$) over dosing interval, AUC of steady state over dosing interval ($AUC_{ss,tau}$), peak concentration at steady state ($C_{max,ss}$) are shown by doses in a table as a result of a simulation.

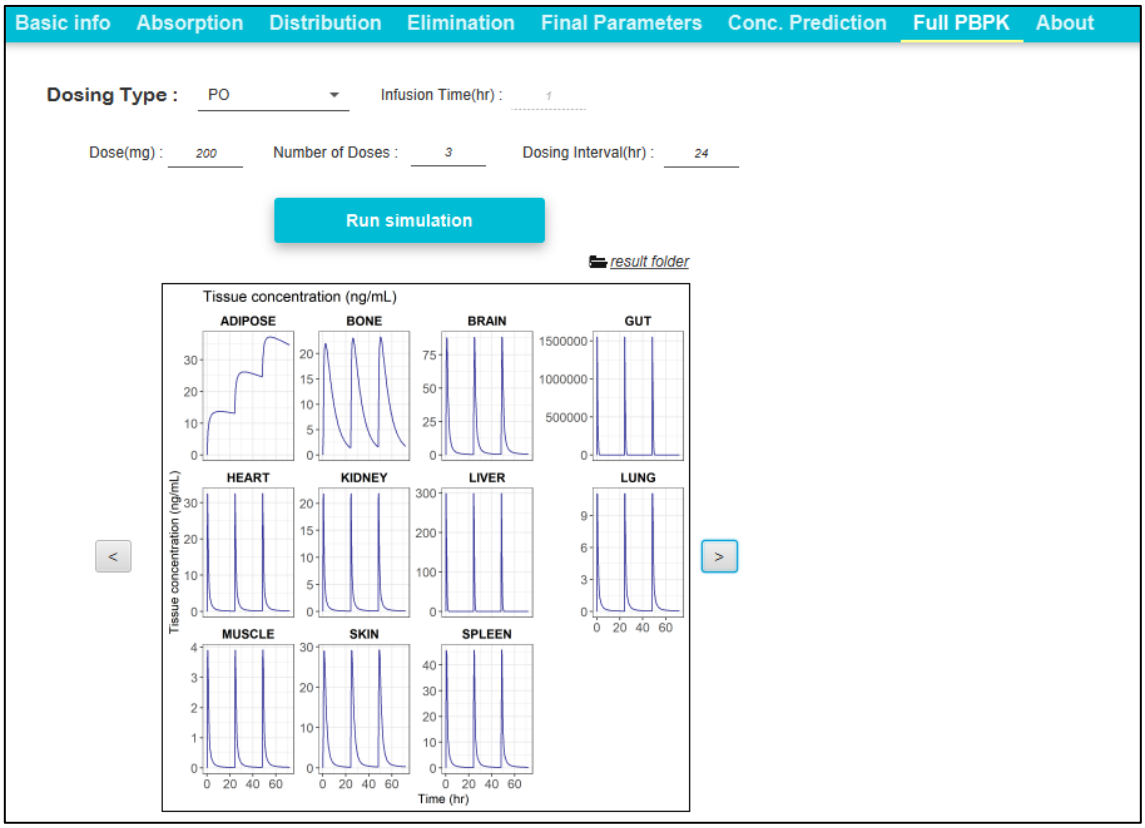
10. Full PBPK (versions ≥ 0.9.0)

1) The full PBPK model is implemented and users can obtain simulated tissue concentration profiles and values. (Based on perfusion rate-limited kinetics using predicted human tissue Kp values[20] shown in the 'Distribution' tab.)

2) Human plasma and tissue concentration-time curves after oral or i.v. dosing are simulated using the dose, interval, and the number of doses input by the user, and the PBPK parameters.

3) Results (simulated datasets), figures and input parameters are also saved in the result folder (Click 'result folder').

4) As current PBPK model is developed based on hepatic CL_{int} and renal CL, simulation is not performed when the user does not enter hepatic CL_{int} value.


Basic info
Absorption
Distribution
Elimination
Final Parameters
Conc. Prediction
Full PBPK
About
Dosing Type :
PO
Infusion Time(hr) :
1
Dose(mg) :
200
Number of Doses :
3
Dosing Interval(hr) :
24
Run simulation
result folder
Tissue concentration (ng/mL)
ADIPOSE
BONE
BRAIN
GUT
HEART
KIDNEY
LIVER
LUNG
MUSCLE
SKIN
SPLEEN
Tissue concentration (ng/mL)
Time (hr)
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Release note (0.9.4)

1. Erroneous simulation of i.v. infusion was corrected in the “Conc. Prediction” tab.
2. The unit of Vdss in the “Conc. Prediction” tab was corrected.
3. Both Fa(Caco) and Fa(Caco, ki) are presented in the “Final Parameters” tab.

Release note (0.9.3)

1. Notes were added for the details of the parameters.
2. Conc. Prediction Tab: The values of the AUC of a single dosing ($AUC_{single\ dose,tau}$) over dosing interval, AUC of steady state over dosing interval ($AUC_{ss,tau}$), peak concentration at steady state ($C_{max,ss}$) were added.
3. ‘CLother’ was added in the Elimination tab to allow users to input intrinsic hepatic CL values according to their prior knowledge or information.
4. Some parameter names were changed for clarification. (Check the abbreviation section in manual!)
5. Hepatocyte surface area calculation method used to predict passive diffusion CL was fixed.

Release note (0.9.2)

1. Erroneous simulation due to inconsistent units of final parameters was fixed.

Release note (0.9.1)

1. User Interface of Elimination tab is redesigned.
2. Elimination Tab: F_h is calculated automatically using $CL_{h,int}$ value according to the dispersion model.
3. Minor bugs (weight units of animal species, units of plot axis, etc) are corrected.

Release note (0.9.0)

1. The completely redesigned user interface (UI) and new features are introduced.
2. Conc. Prediction tab: The simulation speed for concentration prediction has been substantially improved by adopting the mrgsolve R package.
3. Full PBPK tab: The new feature implementing the simulation of plasma and tissue concentration profiles using a full PBPK model is available.
4. The PBPK approach in the Distribution tab: The predicted human tissue distribution (Kp) values in various tissues instead of V_{ss} values are presented to the user.

Release note (0.8.9)

1. Allometric approach in the Elimination tab: the unit of CL (L/hr) was corrected to “L/hr/kg”, and the same unit was marked in the "Allometric scaling result" box.

2. Allometric approach in the Distribution tab and the Elimination tab: When the brain weight (BrW) or maximum life span (MLP) was chosen, the errors of omitting BrW and MLP at the final calculation formulas for Q and CL were corrected.

3. “Hepatocyte” in the “in vitro/in vivo” option of the Elimination tab: the title of the fraction “Vcell/Vincubation” was corrected to “Ncell/Vincubation”.

4. The human body weight fixed to 70 kg was revised so that the user may choose between 60 and 70 kg.

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 V_{ss} 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 ka 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.

Release note (0.8.7)

1. The Qgut model was included to predict the intestinal bioavailability (Fg)
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.

11. References

1. Yim, D.S., S. Choi, and S.H. Bae, *Predicting human pharmacokinetics from preclinical data: absorption.* Transl Clin Pharmacol, 2020. **28**(3): p. 126-135.
2. Yim, D.S. and S. Choi, *Predicting human pharmacokinetics from preclinical data: volume of distribution.* Transl Clin Pharmacol, 2020. **28**(4): p. 169-174.
3. Yim, D.S., S.H. Bae, and S. Choi, *Predicting human pharmacokinetics from preclinical data: clearance.* Transl Clin Pharmacol, 2021. **29**(2): p. 78-87.
4. Palm, K., et al., *Polar molecular surface properties predict the intestinal absorption of drugs in humans.* Pharm Res, 1997. **14**(5): p. 568-71.
5. Usansky, H.H. and P.J. Sinko, *Estimating human drug oral absorption kinetics from Caco-2 permeability using an absorption-disposition model: model development and evaluation and derivation of analytical solutions for k(a) and F(a).* J Pharmacol Exp Ther, 2005. **314**(1): p. 391-9.
6. Sun, D., et al., *Comparison of human duodenum and Caco-2 gene expression profiles for 12,000 gene sequences tags and correlation with permeability of 26 drugs.* Pharm Res, 2002. **19**(10): p. 1400-16.
7. Krishna, R. and L. Yu, *Biopharmaceutics applications in drug development*. 2007: Springer Science & Business Media.
8. Gertz, M., et al., *Prediction of human intestinal first-pass metabolism of 25 CYP3A substrates from in vitro clearance and permeability data.* Drug Metab Dispos, 2010. **38**(7): p. 1147-58.
9. Obach, R.S., et al., *The prediction of human pharmacokinetic parameters from preclinical and in vitro metabolism data.* J Pharmacol Exp Ther, 1997. **283**(1): p. 46-58.
10. Choi, G.W., Y.B. Lee, and H.Y. Cho, *Interpretation of Non-Clinical Data for Prediction of Human Pharmacokinetic Parameters: In Vitro-In Vivo Extrapolation and Allometric Scaling.* Pharmaceutics, 2019. **11**(4).
11. Ring, B.J., et al., *PhRMA CPCDC initiative on predictive models of human pharmacokinetics, part 3: comparative assessement of prediction methods of human clearance.* J Pharm Sci, 2011. **100**(10): p. 4090-110.
12. Bonate, P.L. and D.R. Howard, *Pharmacokinetics in drug development*. 2004: Springer.
13. Berezhkovskiy, L.M., *Volume of distribution at steady state for a linear pharmacokinetic system with peripheral elimination.* J Pharm Sci, 2004. **93**(6): p. 1628-40.
14. Kilford, P.J., et al., *Hepatocellular binding of drugs: correction for unbound fraction in hepatocyte incubations using microsomal binding or drug lipophilicity data.* Drug Metab Dispos, 2008. **36**(7): p. 1194-7.
15. Poulin, P. and F.P. Theil, *Development of a novel method for predicting human volume of distribution at steady-state of basic drugs and comparative assessment with existing methods.* J Pharm Sci, 2009. **98**(12): p. 4941-61.
16. Roberts, M.S. and M. Rowland, *A dispersion model of hepatic elimination: 1. Formulation of the model and bolus considerations.* J Pharmacokinet Biopharm, 1986. **14**(3): p. 227-60.

17. Li, R., et al., *Permeability comparison between hepatocyte and low efflux MDCKII cell monolayer.* AAPS J, 2014. **16**(4): p. 802-9.
18. Naritomi, Y., et al., *Prediction of human hepatic clearance from in vivo animal experiments and in vitro metabolic studies with liver microsomes from animals and humans.* Drug Metab Dispos, 2001. **29**(10): p. 1316-24.
19. Paine, S.W., et al., *Prediction of human renal clearance from preclinical species for a diverse set of drugs that exhibit both active secretion and net reabsorption.* Drug Metab Dispos, 2011. **39**(6): p. 1008-13.
20. Jones, H. and K. Rowland-Yeo, *Basic concepts in physiologically based pharmacokinetic modeling in drug discovery and development.* CPT Pharmacometrics Syst Pharmacol, 2013. **2**(8): p. e63.

12. Abbreviation list

Atenolol Papp: Caco-2 permeability of atenolol

B/P: Blood-to-plasma partition ratio

BrW: Brain weight

Caco-2 Papp: Caco-2 permeability

CL_h: Hepatic clearance

CL_{int}: In vitro intrinsic clearance in microsome/hepatocyte

$CL_{int,cyp3A}$: Intrinsic clearance by liver microsomal CYP3A4

$CL_{int,g}$: Intrinsic clearance by intestinal microsome

CL_{other}: extra intrinsic hepatic CL according to prior knowledge

$CL_{passive}$: Passive diffusion clearance across hepatocyte membranes predicted by MDCK-II permeability

CL_r: Renal clearance

$CL_{r(dog)}$: Renal clearance in dogs

$CL_{r(from\ dog)}$: Human CLr calculated from dog CLr using allometry

$CL_{r(from\ rat)}$: Human CLr calculated from rat CLr using allometry

$CL_{r(rat)}$: Renal clearance in rats

CL_{tot}: Total clearance

$CL_{tot(BrW)}$: CLtot estimated using brain weight corrected allometry

$CL_{tot(mlp)}$: CLtot estimated maximum life-span potential corrected allometry

$CL_{tot(simple)}$: CLtot estimated using simple allometry

F: Bioavailability

F_a: Fraction absorbed

$F_{a(Caco\text{-}2)}$: Fa predicted using Caco-2 permeability

$F_{a(PSA)}$: Fa predicted using polar surface area

F_g: Fraction escaping gut clearance

$F_{g(HIM)}$: Fg predicted using intrinsic CL by intestinal microsome

$F_{g(HLM)}$: Fg predicted using intrinsic CL by liver microsomal CYP3A

F_h: Fraction escaping hepatic clearance (1 – CLh/Qh)

f_u: Plasma unbound fraction in human

f_{u_hep}: fraction of unbound drug in the in vitro hepatocyte incubation

f_{u_mic}: fraction of unbound drug in the in vitro microsomal incubation

K_p: Tissue-plasma partition coefficient

LogD: Octanol:buffer distribution coefficient

LogP: Neutral species octanol:buffer partition coefficient

Method 1: The general IVIVE approach that is obtained using MPPGL and liver weight

Method 2: The general IVIVE approach (method 1) was calibrated by in vivo clearance observed in rats.

Method 3: The general IVIVE approach (method 1) was calibrated by passive diffusion clearance estimated using Caco-2 permeability.

Method 4: The general IVIVE approach (method 1) was calibrated by passive diffusion clearance estimated using log D.

MLP: Maximum life-span potential

P_{app}: Apparent permeability

$P_{app\ (MDCK)}$: MDCK-II permeability

P_{eff}: Effective permeability in intestine predicted by Caco-2 cell permeability

PSA: Polar surface area

Q: Inter-compartmental clearance

$Q_{(BrW)}$: Q estimated using brain weight corrected allometry

$Q_{(MLP)}$: Q estimated using maximum life-span potential corrected allometry

$Q_{(simple)}$: Q estimated using simple allometry

V_c: Central volume

V_p: Peripheral volume

V_{ss}: Volume of distribution at steady state

WT: Body weight