

## Introduction to DallphinAtoM (ver. 0.9.8.5)

### 1. What is DallphinAtoM?

- DallphinAtoM is the acronym of the phrase 'Drugs with **ALL**ometry and **PH**ysiology **I**nside - **A**nimal **t**o **h**u**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 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 0.9.8.5 is distributed to beta testers for test use only.
- Any suggestion, comments or bug reports via email ([yimds@catholic.ac.kr](mailto:yimds@catholic.ac.kr)) or Github Issues (<https://github.com/pipetcpt/dallphin/issues>) are welcomed.

### 4. Who developed DallphinAtoM?

- DallphinAtoM 0.9.8.5 was developed by researchers of PIPET of the College of Medicine, the Catholic University of Korea for the education of students and researchers.
- DallphinAtoM 0.9.8.5 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 0.9.8)



- 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.
- Users can save the current environment and a Microsoft Excel file (xlsx) will be generated in the Result folder. Users also may load the saved xlsx file to resume their environment. (versions  $\geq 0.9.6$ )

Basic info		Absorption	Distribution	Elimination	Final Parameters	Conc. Prediction	Full PBPK	About
<b>Physicochemical Properties</b>								
<div>Neutral</div> <div>default value</div>								
Log P	5							
pKa	7.7							
Log D7.4	Prediction	5						
<input checked="" type="checkbox"/> PSA	200 $\text{\AA}^2$							
<b>Plasma Protein Binding</b>								
<input checked="" type="checkbox"/> fu(rat)	0.2							
<input checked="" type="checkbox"/> fu(dog)	0.4							
fu	0.8							
B/P	1.0							
<b>Note</b> <p>Log P: Neutral species octanol:buffer partition coefficient            Log D: Octanol:buffer distribution coefficient            PSA: Polar surface area            fu: Plasma unbound fraction in human            fu(rat): Plasma unbound fraction in rat            fu(dog): Plasma unbound fraction in dog            B/P: Blood-to-plasma partition ratio</p>								

### 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  $F_{a\_PSA}$ .

## 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 $F_a$

- 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 ( $21.4 \times 10^{-6}$  cm/sec) and atenolol ( $0.37 \times 10^{-6}$  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  $F_a(Caco)$  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  $F_a(Caco, k_i)$  in the "Final Parameters" tab.
- Thus, once the Caco-2 permeability value of the test drug is provided, both  $F_a(Caco)$  and  $F_a(Caco, k_i)$  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 intrinsic clearance by liver/intestinal microsomal CYP3A is not provided.

### 3) MDCK permeability

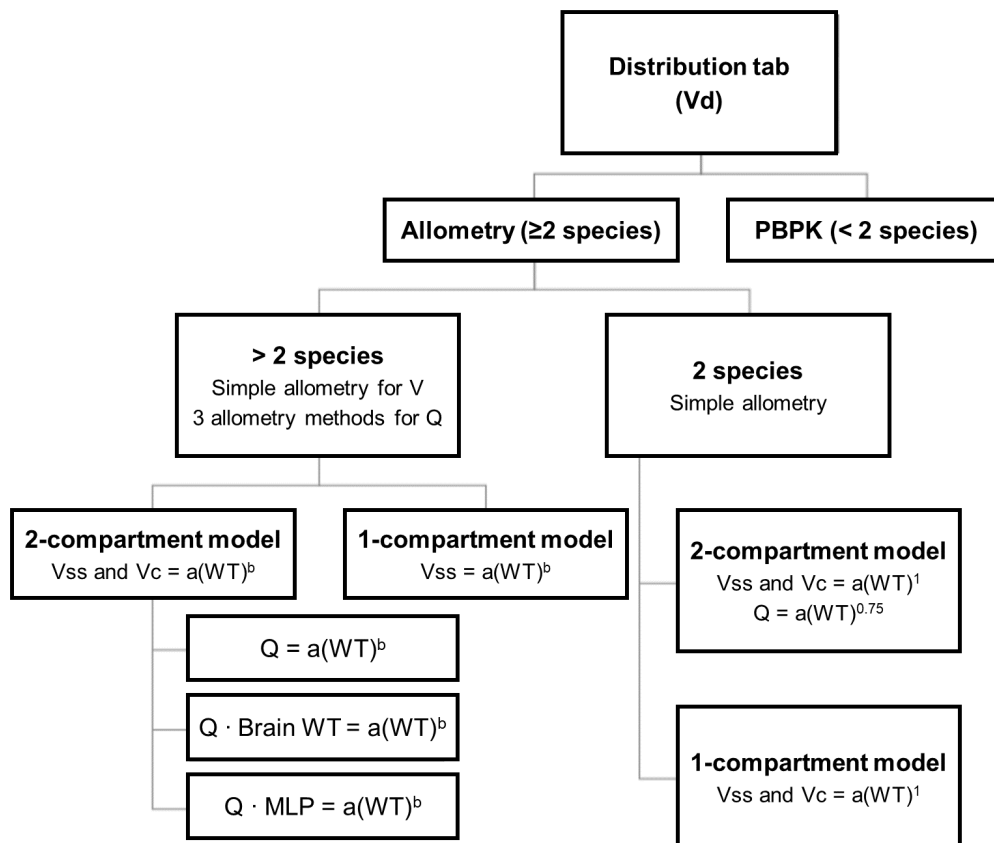
- MDCK-II cell permeability may be input or can be predicted using Caco-2  $P_{app}$ . It is used to estimate passive CL that may be used when the microsomal method is chosen for the calculation of hepatic CL (Elimination tabpane).

### 4) first-order absorption rate constant: $k_a$

- We used an equation to predict human  $k_a$  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  $V_d$  in DallphinAtoM. The user should opt for either of the two methods.
- Background and methods to predict the human volume of distribution ( $V_d$ ) 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  $\geq 3$  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 ( $V = a(WT)^b$ ,  $R^2 = \text{coefficient of determination}$ ) 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  $\geq 3$  is not acceptable.)

Basic info Absorption **Distribution** Elimination Final Parameters Conc. Prediction Full PBPK About

Select Human WT : 70kg

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

**Allometric approach**  
Enter distribution data in animals Two-Compartment Model

Species	Weight	Q(L/hr/kg)	Vc(L/kg)	Vp(L/kg)	Vss(L/kg)	bw	mlp
<input checked="" type="checkbox"/> Mouse	20 g	0.8	0.5	1.0	1.5	0.00036	2.7
<input checked="" type="checkbox"/> Rat	200 g	0.8	0.5	1.0	1.5	0.0018	4.7
<input checked="" type="checkbox"/> Monkey	3.0 kg	0.8	0.5	1.0	1.5	0.09	22.3
<input checked="" type="checkbox"/> Dog	10.0 kg	0.8	0.5	1.0	1.5	0.08	19.7
<input type="checkbox"/> others	0 kg	0	0	0	0.0	0	0

Prediction

**Allometric Scaling Result**

WT 70kg Vc output Vp output Vss output  
Q(simple) output Q(bw) output Q(mlp) output

Note ☒

BW: Brain weight  
MLP: Maximum life-span potential  
Vc: Central volume  
Vp: Peripheral volume  
Q: Inter-compartmental clearance  
Vss: Volume of distribution at steady state  
Q(simple): Q estimated using simple allometry

(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  $\geq 3$  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

**Basic info** Absorption **Distribution** Elimination Final Parameters Conc. Prediction Full PBPK NCA About

Select Human WT : 70kg

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

**PBPK approach**

**Predicted human tissue distribution (Kp)** Pouline & Theil

Bone	8.9318	Lung	
Brain	7.4978	Musc	
Gut	6.4929	Skin	
Heart	11.319	Splee	
Kidney	4.9722	Adipo	
Liver	5.4123		

**Calculation**

	WT	VSS
Human	70kg	60.68 L/kg

**Note**

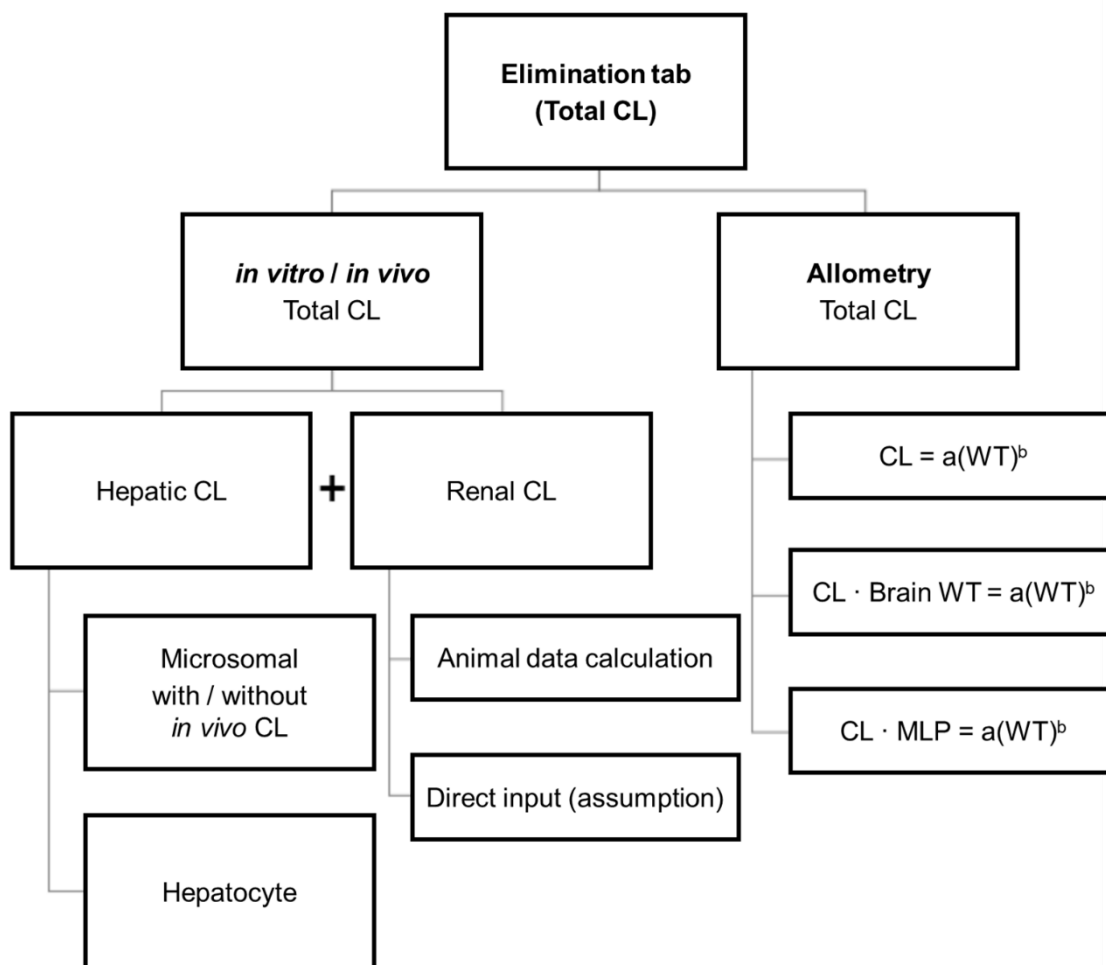
Kp : Tissue-plasma partition coefficient  
Vss : Volume of distribution at steady state

- 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. Users can select one of several methods for  $K_p$  values, and the description can be found in the webpage of KpCoeff R package.<sup>1</sup>
- 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.

<sup>1</sup> KpCoeff is an R package for prediction of tissue-plasma partition coefficients ( $K_p$ ) using published method with published/unified dataset. <https://github.com/sueinchoi/KpCoeff>



## 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.



☒ **in vitro / in vivo**

**Hepatic Clearance(CL<sub>h</sub>)**

☒ Microsome

CL<sub>int</sub> : ☒ Rat 125 ul/min/mg protein      CL<sub>int, rat(in vivo)</sub> 25 ml/min/kg

Human 45 ul/min/mg protein

microsome protein concentration 1.0 mg/mL

fu<sub>mic</sub> Prediction 0.0892

CL<sub>h</sub>(Method 1) 80.7066 L/hr      CL<sub>h</sub>(Method 2) 77.8130 L/hr

- The microsome protein concentration should also be input.
- Three kinds of CL (Method 1,3,4) are automatically calculated from the CL<sub>int</sub>. 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) **CL<sub>h</sub>(Method1)**: In the microsome experiment, when the user inputs microsome intrinsic CL (CL<sub>int</sub>), 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<sub>h</sub>).
- (2) **CL<sub>h</sub>(Method3)**: As intrinsic CL can be over-predicted in microsome experiments, passive diffusion CL is applied to intrinsic metabolic CL (CL<sub>int</sub>) to reflect the diffusion rate of drug molecules across the hepatocyte cell membrane.
  - Passive diffusion CL is calculated using the apparent passive permeability (P<sub>app</sub>) of MDCK-II cell (the information input in the Absorption tabpane) and surface area of human hepatocytes. [17]
  - When the P<sub>app</sub> of MDCK-II is not available, P<sub>app</sub> of Caco-2 is used instead. A linear correlation exists between the two P<sub>app</sub>, 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 P<sub>app</sub>s: correlation between logD and P<sub>app</sub> of MDCK-II is used. [17] Then, the CL<sub>h</sub> is denoted as "CL<sub>h</sub>(Method4)" in the final parameters tabpane.
- (3) **CL<sub>h</sub>(Method2)**: The CL<sub>h</sub> is also corrected using rat in vitro-in vivo correlation (ivivc) data with the "rat" checked as below.).

## ✓ in vitro / in vivo

### Hepatic Clearance(CL<sub>h</sub>)

#### ○ Microsome

CL<sub>int</sub> : ☒ Rat 125 ul/min/mg protein

When both of rat microsomal metabolism data (CL<sub>int</sub> of the rat) and rat *in vivo* hepatic CL (CL<sub>h, rat</sub>(in vivo)) are available, their ratio may be applied to obtain corrected human hepatic CL. [18]

- A. First, corrected CL<sub>int</sub> of rat (CL<sub>int, corrected</sub>) is back-calculated from observed CL<sub>h</sub> of rat (CL<sub>h, rat</sub>(in vivo)) using the well-stirred model.

$$CL_{-rat\,in\,vivo} = \frac{Q \times f_u \cdot CL_{int, corrected}}{Q + f_u \cdot CL_{int, corrected}} \Rightarrow CL_{int, corrected} = ?$$

- B. Second, the ratio between the CL<sub>int</sub> and CL<sub>int, corrected</sub> in the rat is calculated.
- C. Third, the ratio is used to calculate the human's CL<sub>int, corrected</sub>.
- D. Last, human CL<sub>int, corrected</sub> is used to calculate CL<sub>h</sub>(Method 2) using the well-stirred model equation.
- (4) Correction using rat *ivivc* is not provided for CL<sub>h</sub>(Method 3) or CL<sub>h</sub>(Method 4) because experiments on rat hepatocyte permeability are not generally done.

## 2) when using human hepatocyte data

☒ Hepatocyte

CL<sub>int, ini</sub> : Human 115 ul/min/10<sup>6</sup> cell

N<sub>cell</sub> / V<sub>incubation</sub> 0.005

fu<sub>hep</sub> Prediction ▼ 0.1354

CL<sub>h</sub> 85.662 L/hr

- The hepatocyte cell volume ratio can be input considering user's experimental condition. Generally, it is considered to be 0.005 when 10<sup>6</sup> hepatocyte cells were used.
- 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 CL<sub>h</sub>(Method1).
- The CL<sub>h</sub>(Method2) is not provided because *in vitro* metabolism study using rat hepatocytes is rarely performed.
- The CL<sub>h</sub>(Method 3, 4) is not provided because passing through the hepatocyte cell membrane was already reflected to the CL<sub>int</sub> obtained from the human hepatocyte experiment.

## 6. Renal CL in the Elimination tab

- 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<sup>2</sup> 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) ( $CL \times \text{MLP} = a(WT)^b$ )
- 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.

☒ **allometric approach**

**Total Clearance**  
 Enter clearance data in animals

Species	Weight	CL(L/hr/kg)	bw	mlp
<input checked="" type="checkbox"/> Mouse	20 g	0.8	0.00036	2.7
<input checked="" type="checkbox"/> Rat	200 g	0.8	0.0018	4.7
<input checked="" type="checkbox"/> Monkey	3.0 kg	0.8	0.09	22.3
<input checked="" type="checkbox"/> Dog	10.0 kg	0.8	0.08	19.7
<input type="checkbox"/> others	0 kg	0	0	0

Prediction

**Allometric Scaling Result**  

WT	70kg
CLtot(simple)	0.8 L/hr/kg
CLtot(bw)	0.48 L/hr/kg
CLtot(mlp)	0.42 L/hr/kg

## 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<sub>h</sub>, renal CL, and F<sub>a</sub> 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  $CL_h$  and  $F_h$ .
- 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.

Basic info Absorption Distribution Elimination **Final Parameters** Conc. Prediction Full PBPK About

Prediction in a 70-kg human

☒ in vitro / in vivo

**Hepatic CL**

☒ CL<sub>h</sub>(method 1) 80.7066 L/hr

☐ CL<sub>h</sub>(method 2) 77.8130 L/hr

☐ CL<sub>h</sub>(method 3) 17.1502 L/hr

☐ CL<sub>h</sub>(method 4) 24.5021 L/hr

**Renal CL**

☒ CL<sub>r</sub> (from rat) 18.0923 L/hr

☐ CL<sub>r</sub> (from dog) 12.8290 L/hr

☐ CL<sub>r</sub> (user input) 0.0 L/hr

☐ allometric approach

**Total Clearance**

☒ CL<sub>tot</sub>(simple) output L/hr/kg

☐ CL<sub>tot</sub>(bw) output L/hr/kg

☐ CL<sub>tot</sub>(mlp) output L/hr/kg

Note ☐

CL : 98.8 L/hr

F : 0.06208

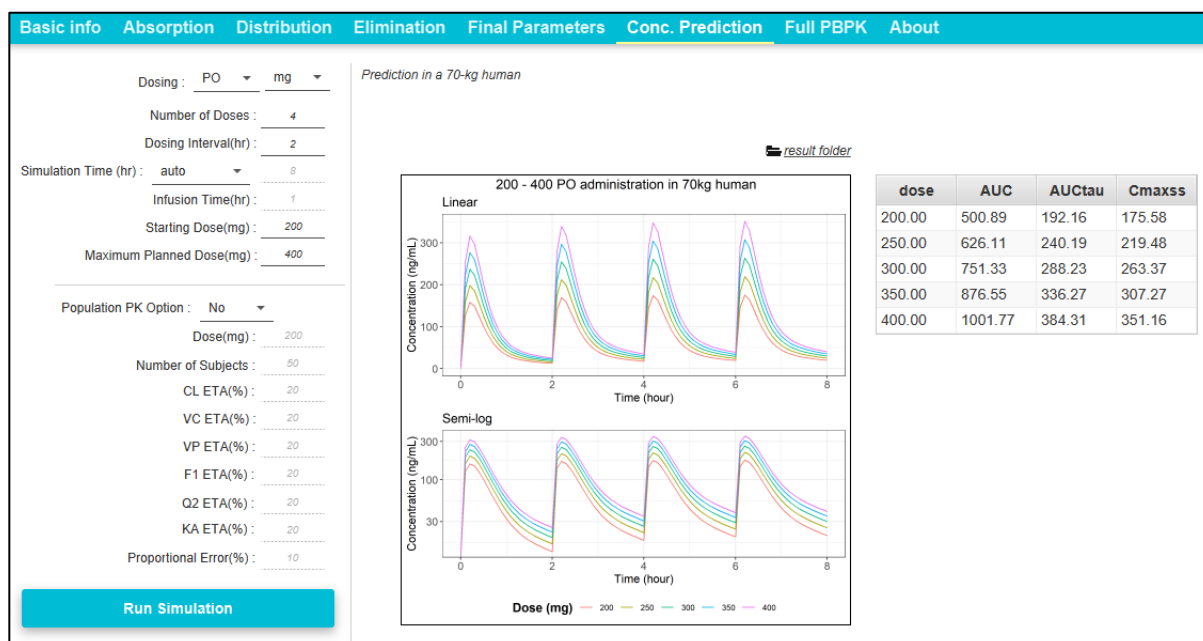
Q : output

V<sub>ss</sub> : output

V<sub>c</sub> : output

K<sub>a</sub> : 2.13 /hr

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

## 10. Full PBPK (versions $\geq 0.9.0$ )

- 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  $CL_{\text{int}}$  and renal CL, simulation is not performed when the user does not enter hepatic  $CL_{\text{int}}$  value.



## 11. NCA tab (versions $\geq 0.9.6$ )

- 1) Noncompartmental analysis (NCA) is a primary analytical approach for PK studies.
- 2) DallphinAtoM performs NCA using the NonCompart R package<sup>2</sup> [21], that was validated to reproduce the NCA results by WinNonlin®.
- 3) When users input their datasets based on the provided Example, the preview of the input file will be shown.
- 4) Calculation methods (methods to calculate AUC using either 'linear-up linear-down' or 'linear-up log-down'), administration route, dosing amount, and the units of the values should be properly entered.
- 5) Results (NCA parameters) can be filtered and saved in the result folder (Click 'Result file path').

<sup>2</sup> NonCompart is an R package to conduct a noncompartmental analysis with industrial strength.  
<http://cran.r-project.org/web/packages/NonCompart>

Basic Info Absorption Distribution Elimination Final Parameters Conc. Prediction Full PBPK **NCA** About

CSV Filepath: C:\DallphinAtoM\R\code\sampladata.csv

Example

Browse

#### Calculation method

- ☒ Log  
☐ Linear

#### Administration route

- ☒ Oral or Extravascular  
☐ Bolus IV  
☐ IV Infusion

Infusion Time(/hr): 0

Dose: 100 mg

Time unit: h

Conc unit: mg/L

NCA calculate

#### Input file summary

Subject	Dose	Time	Conc
1	4.02	0	0.74
1	4.02	0.25	2.84
1	4.02	0.57	6.57
1	4.02	1.12	10.5
1	4.02	2.02	9.66
1	4.02	3.82	8.58
1	4.02	5.1	8.36

#### Result

filter

C <sub>MAX</sub>	T <sub>MAX</sub>	LAMZHL	AUCLST	AUCIFO	VZFC
10.5	1.12	14.304377571097	147.234748537004	214.92363157523	9.601947
8.33	1.92	6.65934156262252	88.7312754883266	97.3779346315098	9.866094
8.2	1.02	6.76608737718236	95.8781977933781	106.127668533925	9.197790
8.6	1.07	6.98124666099893	102.633623210553	114.216204638156	8.818197
11.4	1	8.0022640410078	118.179353752805	136.304731589923	8.469864
6.44	1.15	7.89499786796582	71.6970149943727	82.1758833245604	13.86060
7.88	0.48	7.81666866188418	87.668871857558	108.88768888818	11.88888

Result file path : c:\DallphinAtoM\results\nca\2022-08-19-09-58-31\result.csv

## 12. Release note

### Release note (0.9.8.5)

1. Minor changes (default uncheck status and order of data input) in the Basic Info tab were given to avoid confusion.

### Release note (0.9.8)

1. The error in the PBPK method section in the Distribution tab was corrected.

### Release note (0.9.7)

1. The calculation and simulation speed has been improved.
2. In NCA tab, the digit of numbers is fixed.
3. Minor bugs are corrected.

### Release note (0.9.6)

1. Noncompartmental analysis is possible (NCA tab).
2. In Distribution tab, more Kp calculation methods can be selected.
3. A save/load feature is available using a Microsoft Excel file (xlsx).

### Release note (0.9.5)

1. In Elimination tab, previously, Hepatic CL of CLh (Method2) based on microsome assay was calculated only with Rat data, now Dog and Monkey data can be used.
2. In Basic info tab, a field to receive the fu\_monkey value required for calculation was added.
3. In Elimination tab, the fu\_mic and fu\_hep values were not properly reflected in the CLh and Fh result values.

### 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 ( $AUC_{\text{single dose}, \tau}$ ) over dosing interval, AUC of steady state over dosing interval ( $AUC_{ss, \tau}$ ), peak concentration at steady state ( $C_{\text{max}, ss}$ ) were added.



3. 'CLoother' 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 ( $K_p$ ) 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  $k_a$  and  $F_a(\text{Caco})$  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 ( $F_g$ )
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  $F_a(\text{Caco})$  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.

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#### 14. 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<sub>h</sub>: Hepatic clearance

CL<sub>int</sub>: In vitro intrinsic clearance in microsome/hepatocyte

CL<sub>int,cyp3A</sub>: Intrinsic clearance by liver microsomal CYP3A4

CL<sub>int,g</sub>: Intrinsic clearance by intestinal microsome

CL<sub>other</sub>: extra intrinsic hepatic CL according to prior knowledge

CL<sub>passive</sub>: Passive diffusion clearance across hepatocyte membranes predicted by MDCK-II permeability

CL<sub>r</sub>: Renal clearance

CL<sub>r(dog)</sub>: Renal clearance in dogs

CL<sub>r(from dog)</sub>: Human CL<sub>r</sub> calculated from dog CL<sub>r</sub> using allometry

CL<sub>r(from rat)</sub>: Human CL<sub>r</sub> calculated from rat CL<sub>r</sub> using allometry

CL<sub>r(rat)</sub>: Renal clearance in rats

CL<sub>tot</sub>: Total clearance

CL<sub>tot(BrW)</sub>: CL<sub>tot</sub> estimated using brain weight corrected allometry

CL<sub>tot(mlp)</sub>: CL<sub>tot</sub> estimated maximum life-span potential corrected allometry

CL<sub>tot(simple)</sub>: CL<sub>tot</sub> estimated using simple allometry

F: Bioavailability

F<sub>a</sub>: Fraction absorbed

F<sub>a(Caco-2)</sub>: F<sub>a</sub> predicted using Caco-2 permeability

F<sub>a(PSA)</sub>: F<sub>a</sub> predicted using polar surface area

F<sub>g</sub>: Fraction escaping gut clearance

F<sub>g(HIM)</sub>: F<sub>g</sub> predicted using intrinsic CL by intestinal microsome

F<sub>g(HLM)</sub>: F<sub>g</sub> predicted using intrinsic CL by liver microsomal CYP3A

F<sub>h</sub>: Fraction escaping hepatic clearance ( $1 - CL_h/Q_h$ )

f<sub>u</sub>: Plasma unbound fraction in human

f<sub>u\_hep</sub>: fraction of unbound drug in the in vitro hepatocyte incubation

f<sub>u\_mic</sub>: fraction of unbound drug in the in vitro microsomal incubation

K<sub>p</sub>: 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

NCA: Noncompartmental analysis

$P_{app}$ : 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